NEW YORK
STATE DEPARTMENT OF HEALTH
Herman E. Hilleboe, M.D.
Commissioner

LABORATORY MANUAL
FOR
PHYSICIANS

Aids in Diagnosis and Treatment

Issued by
DIVISION OF LABORATORIES AND RESEARCH
ALBANY

Gilbert Dalldorf, M.D., Director

1948

Ninth Edition

". . . add to scientific knowledge through an inquiring attitude of mind, a scientific outlook, a zest for truth, a patience for experiment, and a capacity for logical deduction."

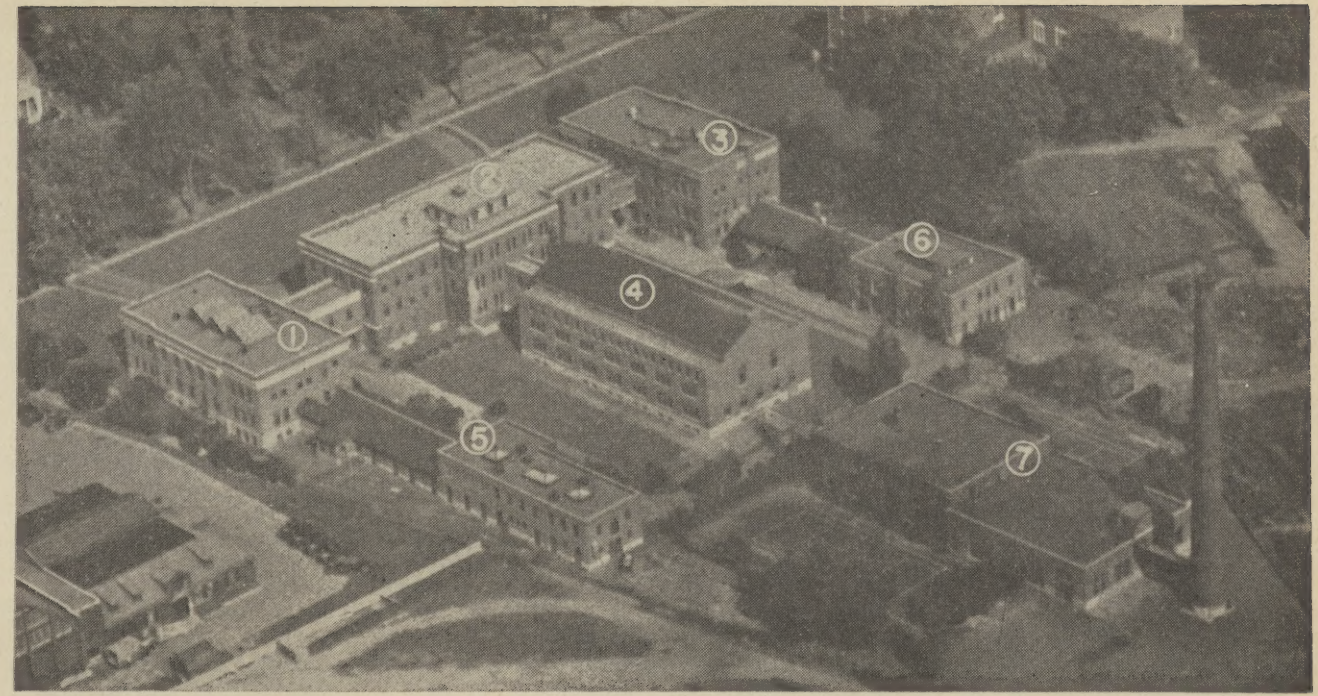
From—Public Health in New York State. Report of the New York State Health Commission to His Excellency, The Honorable Franklin D. Roosevelt, Governor of the State of New York. Albany, 1932. (Committee on Laboratories, Simon Flexner, *Chairman*)

FIGURE 1

DIVISION OF LABORATORIES AND RESEARCH

North Façade Main Building, East and West Wings, New Scotland Avenue, Albany. Occupied successively 1919, 1924, and 1929.

FIGURE 2



DIVISION OF LABORATORIES AND RESEARCH

Airplane View of Laboratories and Auxiliary Structures, New Scotland Avenue, Albany, 1939.

1. West Wing: Antitoxin Serum, and Vaccine Laboratories; 2. Central Building: Administration, General Services, and Research; 3. East Wing: Diagnostic Laboratories; 4. South Wing: Media Department, Library; 5. and 6. Animal Units; 7. Power Plant, Carpenter and Machine Shops.

DIVISION OF LABORATORIES AND RESEARCH

NEW YORK STATE DEPARTMENT OF HEALTH

Central Laboratory, New Scotland Avenue, Albany, 1
Branch Laboratory, 339 East 25th Street, New York, 10

GILBERT DALLDORF, M.D., *Director*
JOHN K. MILLER, M.D., *Associate Director*

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Lilian C. Smith, *Secretary to the Director*

DIVISION OF LABORATORIES AND RESEARCH
NEW YORK STATE DEPARTMENT OF HEALTH

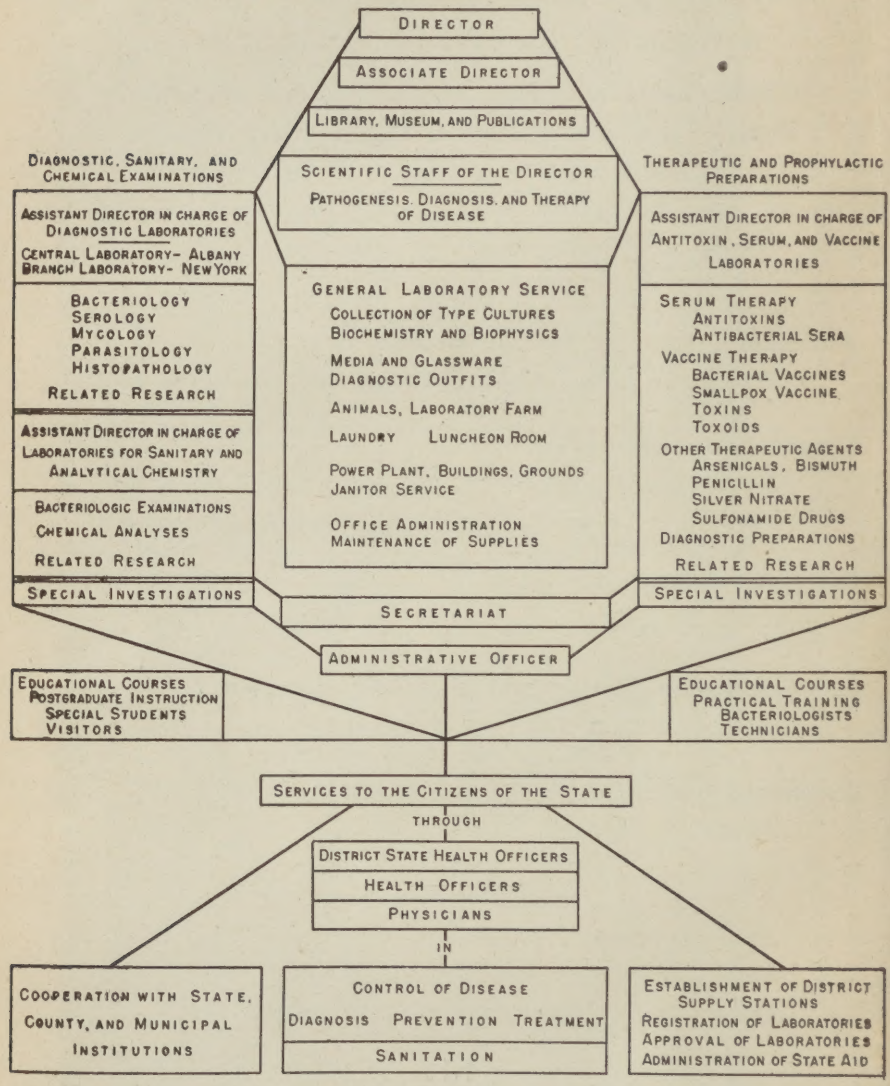

FIGURE 3. CHART OF ORGANIZATION

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INTRODUCTION

The value of laboratory procedures in the diagnosis and control of disease is so firmly established that it is but rarely questioned. It is the hard core of much medical and surgical practice and is relied on daily in decisions of the utmost importance.

One problem has obstinately remained. It is the difficulty of training clinicians and pathologists to work together as effectively as is possible. It is a problem incapable of simple solution or explanation. The physician may not see the patient until the best opportunity for collecting a decisive specimen has passed. The laboratory of necessity frequently changes its technical procedures. The physician may not appreciate the laboratory significance of certain clinical manifestations or not furnish sufficient information to guide the laboratory worker properly.

Pathologists are fully aware of the seriousness of this problem. Too often clinicians are only frustrated. It is not surprising that the New York State Association of Public Health Laboratories has a standing Committee on The Use of Laboratory Facilities. The leaflets distributed by the Association are a useful tool in improving the situation. The *Laboratory Manual* serves a similar purpose. It provides concise, practical information to guide the physician in the collection and handling of materials that require laboratory examination. It also contains essential information concerning his obligations under the Public Health Law and the Sanitary Code.

This edition of the Manual, like its predecessors, has been prepared by the staff of the Division of Laboratories and Research. Each section has been written by the staff member directly responsible for the examinations concerned. There need be no doubt therefore of the reliability and usefulness of the recommendations contained herein.

The present edition differs in many details from the preceding one. More attention has been devoted to those diseases due to filterable viruses. Unfortunately diagnostic services for many of these are not yet available because of the delayed construction program of the Division, a result of the war. It is earnestly hoped that the number for which precise diagnostic procedures are available will shortly increase and a number of diseases have been included in the expec-

tation that laboratory examinations will have become feasible within the lifetime of the present edition.

It is noteworthy that the radical changes in microbiology, the development of biochemical concepts of immunity, bacterial nutrition and growth, and the significant new knowledge of bacterial antagonism and host-parasite relationships have but little affected the basic examinations used as aids in diagnosis. It should also be noted that the continued inclusion of certain diseases that are presently uncommon in New York does not indicate fixed habits of thought but considered judgment based on considerable experience. The diseases in question, notably typhoid fever and diphtheria, are quite capable of plaguing us again if our controls are relaxed. Eternal vigilance is the price of protection. Since clinical skill in the diagnosis of uncommon infections is difficult to maintain it is all the more important that laboratory examination be frequently solicited.

Medical laboratory services in New York State are furnished by 138 local laboratories approved by the Commissioner of Health, and by the Division of Laboratories and Research in Albany and its branch in New York City.

The Division of Laboratories and Research includes sections for diagnostic service, the preparation of prophylactic and therapeutic products, sanitary examinations, and research. Diagnostic tests are provided for areas of the State without local service and for the affiliated laboratories whenever the examination is an unusual one for which the laboratory is not equipped or has not been approved, or when confirmatory examinations are desired. The therapeutic and prophylactic preparations are distributed to physicians through 198 supply stations. The sanitary and analytical laboratories primarily serve the State Department of Health and its representatives in problems of stream pollution, water and milk control, and sewage treatment. The research program supports all of the activities of the Division and is directed toward the investigation of fundamental problems and the development and improvement of methods used in examination of specimens and in the preparation of biologic products. The *Standard Methods* of the Division, now in the third edition (Williams and Wilkins Company, Baltimore, 1947, 990p.), describes the technics employed.

The local laboratories are approved for examinations that have public importance. Approval is based on the qualifications and demonstrated ability of the director, the adequacy of the plant, and agreement by the director that the work of the laboratory will be

conducted by approved methods and that standards will be maintained which insure the reliability of results. Thirty-eight laboratories receive state aid. These laboratories have been organized by cities or counties and operate under boards of managers representative of the interests involved.

In their extent and completeness the laboratory services in New York are exceptional. The total number of tests performed in 1947 amounted to more than six million. State-wide collaboration is furthered by the New York State Association of Public Health Laboratories, now in its thirty-second year. The Association has led in the development of laboratory services and sciences. The Association meets twice a year and its printed *Proceedings* reveal the scientific activities of its members.

The laboratory services in New York are closely associated with the hospitals of the State as well as with the public health services. With but few exceptions they are located in hospitals and thereby maintain that intimate connection with clinical medicine that is so important and that has been a principle of the New York system since it was first enunciated by Hermann Biggs.

Modern medicine, like modern public health, cannot operate effectively without adequate laboratory service. Laboratory service cannot remain adequate unless it is constantly refreshed by new discovery and characterized by a spirit of investigation and a determination to advance the bounds of human knowledge. Original work is undertaken by many of the local approved laboratories and has always been a cornerstone of the organization of the central laboratory. The *Collected Studies* of the Division are available to medical libraries, and to interested physicians for reference, as is the *Annual Report* in which considerable research is described. An *Index to the Publications of the Division of Laboratories and Research,* covering the period 1914-1944, has been published separately.

A cardinal principle of the Division has been that laboratory services should be consultative, that close relations are necessary between the physician and the pathologist. This has been the primary reason for the encouragement of local services and the granting of state aid. The central laboratory must rely largely on occasional visits and chiefly on correspondence with physicians. It welcomes these contacts and is also always prepared to receive visitors who wish to see the work of the Division.

PART I

PUBLIC HEALTH LABORATORY SERVICE IN NEW YORK STATE

The Division of Laboratories and Research in its present form began, as a successor to the earlier State Hygienic Laboratory, with the reorganization of the State Department of Health in 1913-1914. With a staff of less than twenty, the work was done in a small frame building and a remodeled stable on Yates Street, in sharp contrast to the modern, well-equipped buildings it now occupies in the pursuit of its statewide activities. In 1919 the laboratory moved into the new main building on New Scotland Avenue; in 1924, the east wing was opened, chiefly for the use of the diagnostic laboratories, and in 1929 the antitoxin, serum, and vaccine laboratories were transferred to the new west wing. In that year a power-house and two new stable units were also constructed. Early in 1939 the south wing, which houses the media department and the library, was occupied. The laboratory farm, within a few miles of Albany, has undergone a parallel development. The plant there now includes a large main unit and five additional buildings for separate phases of the work. This building program offers an excellent illustration of the expansion of the work year by year, for each addition has been a necessary step in the advancement and maintenance of efficient service. Appropriation has now been made for a separate laboratory for work in virology.

There is one branch laboratory of this Division, established in 1914 and now located at 339 East 25th Street, New York, 10, in close proximity to the important medical centers; and a Field Sanitation Laboratory, 295 Oak Street, Buffalo, for the examination of samples of milk from the western section of the State. The approved laboratories scattered throughout the State, serving the larger cities and most of the counties, provide close contact between the health officers and physicians and the central laboratory. Many technical diagnostic procedures must be performed at the bedside or in a laboratory nearby. The close contact thus afforded is an important factor in effective laboratory service; its development supplements the facilities offered by the central laboratory and the branch.

The expanding laboratory service in New York State has presented an interesting problem because of the uneven distribution of the population. The State now has a service that is unique in the extent to which it reaches the citizens of the various districts. The close cooperation between the approved laboratories and the Central Laboratory in Albany ensures uniform methods and reliable results.

This manual outlines the organization and operation of the laboratory service offered by the State, and contains information on how physicians and health officers can avail themselves of it. The Public Health Law and the Sanitary Code should be consulted when there is any doubt concerning legal requirements or provisions.

Approved Laboratories

The function of the laboratory is to assist physicians, health officers, and, through them, the citizens of the State in the prevention, diagnosis, and control of disease and in the maintenance of public health sanitary services. The laboratory must be developed to meet the needs of the district served and physicians and health officers should be thoroughly familiar with the facilities available.

The regulations of the Sanitary Code of the State* require physicians to submit specimens from certain of the communicable diseases, as well as tissue removed at operation or at necropsy that requires laboratory examination as an aid in the diagnosis, prevention, or treatment of disease or to determine the cause of death, to a laboratory approved by the State Commissioner of Health for the examination of such specimens (Chap. II, Reg. 9; Chap. IV, Reg. 7). Specimens of blood from applicants for a marriage license and from pregnant women must also be submitted to an approved laboratory for serologic tests for evidence of syphilis (Domestic Relations Law, Art. III, Sec. 13-a; Public Health Law, Art. II-A, Sec. 18-d). There are approximately one hundred and thirty approved diagnostic laboratories in New York State, outside of the city of New York; they include county, city, hospital, and private laboratories. Only a few of them have not received approval for the examination of specimens of tissue.

The Sanitary Code also requires that health officers have the following sanitary examinations made in approved laboratories:

* The New York State Sanitary Code does not apply to the city of New York. Laboratories there whose directors wish to make official examinations of specimens from patients living outside of the city or to act as consultants in districts where the State Sanitary Code is effective are approved by the state commissioner of health on the same basis as laboratories located elsewhere in the State.

bacterial milk counts for the purpose of grading (Chap. III, Reg. 5), samples of water in connection with control of the sanitary quality of public water supplies (Chap. V, Reg. 3d), and examinations of eating, drinking, and cooking utensils necessary to determine compliance with the Code (Chap. XIV, Reg. 3). Most of the laboratories approved for diagnostic procedures also perform sanitary examinations. In addition, more than fifty laboratories are approved for this type of work only. The Sanitary Code (Chap. III, Reg. 1) requires that bio-assays of vitamin D milk also be made in an approved laboratory. Four laboratories are approved for making such determinations.

A pamphlet in regard to local laboratory service, which includes a list of laboratories and the examinations for which approval has been issued, is distributed annually to registered physicians in the State outside of the city of New York.

When the need for local laboratory service has been realized in a community, the first step is usually the appointment of a committee by the county medical society to ascertain the present and probable demands, the most feasible means by which service may be provided, and the approximate cost. Valuable information and advice may be obtained by consulting the district state health officer. State aid may be secured for county and city laboratories meeting certain requirements. If efficient laboratories are already conveniently located nearby, service may sometimes be procured through contract. Usually, however, service by contract is not as satisfactory as the establishment of a laboratory. The most advantageous location for a laboratory is a general hospital, where the director will be available for consultation with the physicians and surgeons.

Laboratories are maintained under such varying conditions, and the scope of the work and the size of the area served differ so widely that it is difficult to formulate a budget for general application. Salaries of workers depend upon training, experience, and skill. The amount offered must be sufficient to attract individuals competent to undertake the required work satisfactorily. A well qualified director, who must be a graduate in medicine with adequate training in pathology and bacteriology to comply with the provisions of the Sanitary Code, would seldom be attracted by a salary of less than $7,500 per year. The directors of larger laboratories expect from $9,000 to $12,000. If a laboratory serves a large institution or an extended area, the appointment of an associate director is essential. He should be a physician with adequate basic training in pathology

and bacteriology, and a salary of at least $6,000 should be provided. A laboratory serving county or municipal areas must have on the staff a person well trained and qualified in the field of sanitation. Larger laboratories require the services of a trained biochemist, at a salary of $4,000 or more. The number of technicians depends on the scope of the work. Senior technicians with a broad background of experience generally receive from $2,500 to $3,500 per year; less experienced workers, at least $1,500, cleaners and helpers, a minimum of $1,200. Quarters, light, and heat ordinarily are a laboratory expense; when a county or city laboratory is located in a hospital, an estimate is made of the costs to the hospital and this sum is paid by the county or city treasurer directly to the institution. The hospital may, however, provide quarters without charge, in view of the benefits derived. In some instances, money for equipment and maintenance has been provided by public-spirited citizens. Office expenses and the cost of supplies and travel vary considerably. The Division of Laboratories and Research is glad to assist localities or institutions in estimating the probable expense of laboratory service to meet the particular need.

The following are estimates of the probable cost, which varies according to the scope of the work and the size of the district served.

Director (graduate in medicine with adequate training in pathology and bacteriology) ...	$ 8,000	$12,000
Associate or assistant director (graduate in medicine)	6,000	8,000
Medical librarian	2,500	3,500
Chemist	4,000	6,000
Technician	2,000	3,500
Technician	1,800	2,500
Technician	1,500	1,800
Cleaner and helper	1,200	1,500
Cleaner and helper	1,200	1,500
Clerk	1,500	1,800
Secretary or stenographer	1,800	2,500
*Rent, fuel, light, water, etc.	2,500	4,000
Supplies	2,500	6,000
Travel	500	1,000
	$34,500	$52,100
Cost of initial equipment	3,500	6,000
	$38,000	$58,100

* The amount for these items would depend upon the location of the laboratory.

When the necessary data have been secured, the committee appointed by the county medical society should present the facts and the request for an appropriation to cover the desired service to the board of supervisors of the county or the common council of the city.

The Public Health Law (Art. III, Sec. 20-c-h) authorizes boards of supervisors in counties, and the common council or any body exercising similar powers in cities, to establish laboratories or provide laboratory service, toward the support of which state aid may be granted: $2,500 for initial installation and equipment and one-half the cost of yearly maintenance not in excess of $7,500. A laboratory established under this act must have a board of managers consisting of at least five members representing the various interests in the district served, two of whom are physicians licensed to practice in the State. The board of supervisors may confer the powers and duties of the board of managers upon the county board of health if such a board exists.

Before a laboratory can be approved for diagnostic procedures, the director must have the qualifications outlined in the Sanitary Code (Chap. XI, Reg. 18-23).

On January 15, 1937, the Public Health Council adopted the following resolution relating to the approval of laboratories: "Resolved, that, diagnostic laboratory service being intimately concerned with the practice of medicine, the state commissioner of health be advised that a laboratory offering diagnostic service should not be approved unless, in addition to meeting other conditions which may be prescribed, the person actively in charge is licensed to practice medicine or eligible for examination for license to practice medicine in the State of New York."

Procedure of Approval

Approval of a laboratory is considered by the Division of Laboratories and Research after an application has been made by the person in charge, and is issued only in case the applicant has qualifications that meet the requirements prescribed, has demonstrated his ability to perform the duties of the position satisfactorily, and agrees to conduct the work of the laboratory in an ethical manner and to maintain the technical standards required for laboratories approved under the authority of the commissioner of health.

Four years of postgraduate training and experience in the department of pathology of a medical school recognized by the Regents of the University of the State of New York, including training and experience in pathology, bacteriology, and related departments, or an equivalent combination of training and experience have been considered as meeting the

requirements for directors and pathologists outlined in Regulations 21 and 22 of the Sanitary Code.

Directors, pathologists, and bacteriologists shall either be on full time or devote the major part of their time and attention to the work of the laboratory. When they cease active laboratory work for a long period of years, their qualifications must be reviewed in the light of advances in knowledge in bacteriology and pathology that have taken place in the interim, before approval is issued.

Persons in charge of laboratories approved for bacteriologic examinations of milk and water and for examinations concerned with eating, drinking, and cooking utensils shall have had adequate training and experience in the technical procedures involved and, in addition, shall be thoroughly versed in the principles of sanitary science in order that they may be competent to interpret the significance of the laboratory findings. Those in charge of sanitary chemical analyses of water in laboratories approved for this work shall also have had ample training in chemistry, including courses of instruction in analytical chemistry. Persons in charge of laboratories approved for bio-assays of vitamin D milk shall have had adequate training and experience in the technical procedures and shall be competent to interpret the significance of the laboratory findings. Laboratories in which samples of pasteurized milk and cream are examined must be approved also for the phosphatase test to determine pasteurization.

The facilities available, including space, lighting, and equipment, must be adequate for conduct of the work for which approval is desired.

Certificates of approval are issued annually. They are valid until the end of the year in which issued unless sooner revoked. Approval terminates automatically with changes in the personnel in charge of work for which approval has been issued. The laboratory examinations for which approval is granted are specified. New appointees must qualify in the usual manner.

Approval for any type of work may be withheld or withdrawn if examinations are undertaken that are required by the Sanitary Code but for which approval has not been granted, unless duplicate specimens are sent to an approved laboratory for official tests.

Series of specimens are submitted for comparative examination from time to time to the approved laboratories and also upon the request of a director.

A person in charge of a laboratory seeking approval submits a formal application and signs agreements regarding the maintenance of standards of work. The laboratory is inspected by a representative of the Central Laboratory. Applicants for approval in pathology examine a series of sections of tissue that have been selected with special care. Only those sections are used upon which the pathologists of the Roswell Park Memorial Institute in Buffalo and of the Division of Laboratories and Research are in complete agreement

as to the character of the lesion and the suitability of the material for the purpose.

Article 16, Section 185, of the Penal Law as amended in 1947 requires approval by the state commissioner of health for the use of living animals in scientific tests, experiments, or investigations. The term "living animal" has been interpreted to apply only to dogs, cats, horses, guinea pigs, rabbits, mice, and other mammals.

Article XXIII of the Public Health Law requires the registration of places where cultures of pathogenic microorganisms or viruses are handled. This registration does not apply to laboratories maintained by the federal government, state, a municipality or a county. The registration law has no relation to the approval of laboratories, but provides for the compilation of a list of addresses where strains of the incitants of disease are maintained.

PART II

LABORATORY AIDS IN THE DIAGNOSIS AND TREATMENT OF DISEASE

Approved laboratory service has developed to such an extent that it is gradually embracing all types of examinations that are helpful in the diagnosis and treatment of disease. In every case, however, the results of laboratory examinations must be interpreted in the light of clinical observations; the ultimate diagnosis rests with the physician. When local approved laboratories are not available or submission of specimens for confirmatory examination is desirable, the facilities offered by the Division of Laboratories and Research may be utilized. One of the principal functions of the Central Laboratory is to provide a consultation service to the approved laboratories.

Submission of Specimens

Special care should be taken to avoid the possibility of an interchange of specimens before mailing and to ensure their prompt delivery to the laboratory in a satisfactory condition. When the examination desired is especially urgent and should be started immediately, the specimen should be delivered by messenger or sent special delivery.

If studies of a medicolegal nature are desired, the director of the laboratory to which specimens are to be submitted should be consulted in advance to determine whether he is prepared to undertake this type of work. The procedure for handling the volume of specimens received at the Central Laboratory cannot conform with medicolegal requirements and no provision has been made for medicolegal investigation.

Certain other specialized types of work are also not undertaken, and requests for examinations in connection with these problems should be directed as follows: adulteration or other defects in food products, to the New York State Department of Agriculture and Markets, The Governor Alfred E. Smith State Office Building, Albany; industrial hazards, to the Division of Industrial Hygiene and Safety Standards, State Department of Labor, State Office

Building, 80 Centre Street, New York; investigation of suspected foul play, to the Scientific Laboratory of the New York State Police, Bureau of Criminal Investigation, 545 Broadway, Albany; quality of drugs, to the State Board of Pharmacy, Education Building, Albany; insects, pests, or parasites, to the State Entomologist, New York State Department of Education, Education Building, Albany; poisonous plants, to the State Botanist, New York State Department of Education, Education Building, Albany; abortion in cattle, to the Bangs Disease Control Laboratory, New York State Department of Agriculture and Markets, 144 Washington Avenue, Albany; other diseases in domestic animals (other than rabies*) to the New York State Veterinary College, Ithaca.

Specimen Outfits

Every effort has been made to provide suitable outfits so that specimens will reach the laboratories in the best possible condition. The importance of selecting the proper outfit cannot be too strongly emphasized. The labels on the mailing cases used by the Central Laboratory and by many of the approved laboratories are marked to indicate the type of examination desired, as "D" for diphtheria. This not only facilitates the sorting of the specimens at the laboratory, and their distribution to the various groups for examination, but also ensures proper handling when they are received after working hours. For example, a mailing case labeled "D" for diphtheria, if received after 5:00 p. m., is placed in the incubator by the night janitor, together with a record of the time it was received. The reporting of the results of the examination is thus facilitated because there is no delay in incubation.

The Central Laboratory provides outfits designed especially for the collection of specimens to be examined for evidence of diphtheria, enteric diseases, gonorrhea, syphilis, and tuberculosis. Miscellaneous outfits are designed for the collection of material from conditions other than those mentioned. All of these outfits may be secured from the district laboratory supply stations maintained throughout the

* Examinations for evidence of rabies are made in the Division of Laboratories and Research, New York State Department of Health, Albany, and the Branch Laboratory, 339 East 25th Street, New York City. Also, the following laboratories are approved for these examinations: Division of Laboratories, Erie County Health Department, 2100 City Hall, Buffalo; New York City Department of Health, Bureau of Laboratories, Foot of East 15th Street, New York City; Lederle Laboratories, Inc., Veterinary Department, Pearl River; and New York State Veterinary College, Diagnostic Laboratory, Cornell University, Ithaca.

State. In districts where approved laboratory service is available, the supply stations distribute outfits furnished by the approved laboratories for the submission of specimens to them; in addition, a limited number of the State outfits is also available in the event that the physicians wish to submit duplicate specimens to the Central Laboratory.

In order to facilitate handling the large volume of specimens received for serologic tests for evidence of syphilis and to guard against possible delay that may render the specimens unsatisfactory for other desired examinations, the following procedure is recommended in sending specimens either to the Central Laboratory in Albany or the Branch Laboratory in New York:

> Use the outfit with a white label and red lettering marked "V," when blood is to be examined for evidence of syphilis.
>
> When blood from the same patient is also to be subjected to another type of examination, such as an agglutination test for evidence of typhoid fever, submit, if possible, a separate specimen in the appropriate outfit.
>
> If for some reason this cannot be done, always place in the outfit accompanying the specimen two forms giving adequate data relating to the case, one, a white, syphilis history form, the other, a pink, miscellaneous form. A supply of the miscellaneous history forms is available in all laboratory supply stations.

Blood-letting needles. The outfits furnished to physicians by the Division of Laboratories and Research for the submission of specimens for serologic tests contain blood-letting needles. They are not included in outfits sent to clinics and institutions. The needles are expensive and, in order that their distribution may be continued, physicians are asked to return them to the laboratory with the specimens, for reconditioning. A small envelope is furnished with each outfit, in which the needle can be placed after use; sufficient space is provided in the mailing case for returning the envelope with the specimen to the laboratory. Since the blood-letting needles have, in general, proved more satisfactory than syringes for collecting specimens, physicians are urged to cooperate in the maintenance of this service. (See Fig. 8, p. 87.)

Information or history forms. An information or history form and directions for collecting specimens accompany each diagnostic outfit. The physician should give pertinent data on the history form so that it will be possible to determine the types of laboratory examinations that will be most helpful; for example, if a patient has been in the tropics, various tests might need to be made

that would not be undertaken as a routine procedure. Some of the information is required by law; some is needed for the guidance of laboratory workers; all of it, studied collectively with large numbers of records at hand, furnishes valuable data regarding the relative efficiency of the procedures commonly used. Inconvenience and loss of time for the physician, patient, and laboratory worker may result from lack of sufficient information.

To avoid the possibility of an interchange of specimens and information forms, either before mailing or during transit, the identification of the patient should always be written on the culture tube or other specimen container, as well as on the history form. Results of examinations can be reported only when the full name of the patient is given or, in the case of chancroid, gonorrhea, and syphilis, the patient's initials and date of birth. Physicians should take special care to record such data legibly. All information regarding a specimen should accompany it if possible. If a letter is written separately, it should include the identification of the patient, a description of the specimen, the date of collection, and the type of examination desired.

Preparation of Specimens

In the preparation of specimens, it is exceedingly important that certain simple rules and precautions be observed. Directions for the collection and the preparation of specimens that accompany the outfits should be read and followed. Moreover, the person preparing specimens for examination should be so familiar with the appearance of the outfits and material that he will know when they are not satisfactory for use.

The following precautions are among those to be observed: careful packing of specimens to avoid breakage and leakage; the use of medium that is neither liquefied nor dried; in the preparation of cultures, proper application of the swab to the surface of the lesion and thorough inoculation of the medium; careful handling of the swab used in collecting a specimen to prevent its coming in contact with surfaces other than those to be cultured; the preparation of thin films of blood or discharge so that they will be sufficiently translucent for microscopic examination; the submission of sufficient material, as in the case of specimens of blood for serologic tests; the proper care of syringes used in collecting blood, in order to avoid hemolysis of the specimen. The method of preparing thick blood films is described under Malaria, p. 49.

After specimens have been prepared, they should be mailed or delivered to the laboratory promptly, since delay may render them unsuitable or unsatisfactory for examination. If kept at room temperature, blood specimens may become hemolyzed, throat cultures overgrown with contaminating microorganisms, and, in the case of fecal specimens, bacillary incitants of enteric disease may be destroyed by the products of decomposition.

Virus or Rickettsial Diseases. The primary isolation of viruses and rickettsiae requires special handling of the specimens. With few exceptions, notably that of poliomyelitis, the specimens should be immediately frozen and stored in dry ice until examined. The central laboratory should be notified that such specimens are being shipped, or should be consulted on means of collecting and handling unusual specimens.

Serologic tests to demonstrate virus or rickettsial infection necessitate the submission of two or more specimens of whole clotted blood, the first (acute-phase) collected early in the illness (the acute-phase specimen may be held in the refrigerator for a day or two and then either forwarded to the central laboratory or if a diagnosis of nonvirus or nonbacterial disease is made, discarded). The second specimen (convalescent-phase) should be collected three to eight weeks after onset of illness; the optimum time interval varies in different diseases. The serologic test will reveal whether there has been an increase in antibodies. The mere presence of antibodies may be due to previous infection. An increase of specific antibodies during recovery from a disease is, however, convincing evidence of current infection.

When specimens of blood are submitted for serologic tests, a special history form should accompany them, preferably a pink slip designed for miscellaneous types of examinations. Adequate clinical data should be furnished.

Blood Cultures. The isolation of microorganisms from the blood provides a crucial aid in diagnosis and prognosis. In the case of typhoid fever, for example, typhoid bacilli can usually be isolated from the blood before they can be found in the feces or urine, and before agglutinative properties of diagnostic degree can be demonstrated in the serum. Blood cultures are of diagnostic importance when clinical evidence suggests typhoid or paratyphoid fever, pneumonia, meningococcemia, brucellosis, sepis (septicemia) arising from localized infections of various tissues and organs, acute or chronic endocarditis, and any fever of obscure etiology. Repetition

of the blood culture is often necessary. Blood cultural tests can be undertaken most satisfactorily in the local laboratory, where the blood which is collected aseptically can be immediately placed in the various types of media, and incubated promptly aerobically, with increased carbon dioxide in the air, and anaerobically—thus providing optimum conditions for the growth of any type of bacteria. Also, the number of colonies which develop in the plating media indicate the relative number of bacteria which were present in the blood. When local laboratory facilities are not available, blood collected aseptically can be submitted to the Central Laboratory in a sterile tube. The clot can then be cultured when the specimen reaches the laboratory. The outfit with the Manila label, designed for specimens of blood to be examined for evidence of enteric disease, should be used. The examination desired should be requested on the history slip which accompanies the specimen. Blood for cultural examination should be collected before sulfanilamides or antibiotics have been administered, unless the examination can be made in a local laboratory where substances which will neutralize the effects of such bactericides can be used in the culture media into which the freshly collected blood is placed. (See p. 104.)

Postal Laws and Regulations

The observance of certain postal laws and regulations regarding the kind of specimens admitted to the mails, the containers to be used, and directions for packing will expedite delivery (U. S. Postal Laws and Regulations, Section 589). Specimens for laboratory examination may be admitted to the mail only when enclosed in mailing cases constructed in accordance with this regulation. All of the outfits distributed by the Division of Laboratories and Research meet these postal requirements. If specimens are mailed in another manner, there should be written or printed on the outside of every package the words, "Specimen for bacteriologic examination. This package should be pouched with letter mail." The packages are then handled as first-class matter, but are subject to third-class postage rates unless weighing over eight ounces, when the fourth-class rate applies.

PROPHYLACTIC AND THERAPEUTIC PREPARATIONS

Antitoxins, sera, and vaccines are prepared, tested, and distributed by the Division of Laboratories and Research. These preparations may be obtained by physicians from local supply stations.

Certain preparations such as silver nitrate solution, arsenicals and penicillin for the treatment of syphilis and gonorrhea, gas gangrene antitoxin, antivenin, and rabies vaccine, prepared elsewhere, are purchased for distribution. Besides these supplies, the central laboratory prepares for use in the local approved laboratories a large number of sera for diagnostic purposes.

> Under no circumstances are any of the antitoxins, sera, vaccines, or other preparations distributed by this department to be sold. A violation of the above rule will subject the violator to the penalty prescribed by Section 1740 of the Penal Code.

Distribution of Preparations

Provision is made in the Public Health Law (Art. II, Sec. 5) for the establishment of district laboratory supply stations by the Commissioner of Health, who appoints the custodians. The latter may, with approval, designate substations. Stations are maintained and operated in accordance with prescribed rules and regulations. All actual operating expenses and a specific sum per station for custodial services are borne by the localities.

The district supply stations and their substations are so located throughout the State as to afford the greatest facilities to health officers and physicians. Many of the stations are in the approved laboratories. The proper care of the prophylactic and therapeutic preparations in the stations is prescribed and monthly reports are sent to the central laboratory in Albany, giving the name and address of the physician, the kind, amount, and lot number of the material obtained, and whether any was returned unused. The local stations are expected to maintain an adequate supply of routine material, such as diphtheria antitoxin, outfits of silver nitrate solution, etc., to meet the usual needs, and enough of certain products, such as tetanus antitoxin for therapeutic use and antimeningococcus serum, for the initial injections of one or two cases before a fresh supply from the Central Laboratory can be ordered by telephone or telegraph. Still other products that are relatively unstable or seldom used, or new products upon the use of which further data are required before they are released for general distribution, can be secured only upon special request made through the local station or directly to the laboratory in Albany.

Health officers and physicians can be of great assistance in conserving State supplies by limiting their requests to material actually needed for current use, by keeping under proper conditions any

material held for a time, and by returning all unused material promptly to their local supply stations or, in the case of special products, to the central laboratory.

All biologic products should be kept in the dark at a low, even temperature; under no circumstances at room temperature or subject to marked temperature changes. It is inadvisable to use any material that has been frozen; it should be returned with this information. Material that has been kept under improper conditions cannot be relied upon to give satisfactory results. Products should not be used after the return date that is stamped on each package.

Precautions against Anaphylactic Reactions

The injection of horse or rabbit serum, whether concentrated or unconcentrated, may, in rare instances, incite severe or even fatal reactions of an anaphylactic character in highly sensitive persons. Such reactions usually occur in persons who suffer from hay fever, asthma or other allergic conditions, or who have previously received an injection of the corresponding serum. Hence, it is highly important to obtain the previous history and to determine whether a condition of hypersensitivity exists. For this purpose both an intracutaneous and an ophthalmic test are used. The intracutaneous test on account of its greater sensitivity should be selected if only one test is made. Even in persons who fail to react to the tests, intravenous or intraspinous injection of serum may induce severe or fatal reactions. Absence of systemic reactions when skin sensitivity has been demonstrated has also been reported. The possibility of reactions makes caution essential in all serum injections. A syringe containing 1.0 ml. of freshly prepared epinephrine (Adrenalin) solution, 1:1000, should be kept at hand for immediate use.

Moderate or severe reactions characterized by chill and sharp rise in temperature that usually occur within from one-half to one hour after serum injection are not considered anaphylactic. Sometimes they are due to pyrogens in the salt solution used to dilute the serum or in the rubber tubing of injection equipment. This type of reaction rarely requires more than symptomatic treatment unless the hyperpyrexia becomes excessive.

Intracutaneous test. An area on the anterior surface of the forearm is gently cleansed with soap and water, then with alcohol, and 0.1 ml. of a 1:100 dilution of normal horse or rabbit serum in sterile physiologic salt solution is injected intracutaneously. If the injection wheal and the slight surrounding erythema do not increase in size in fifteen minutes, and if no pseudopods develop, the injection of serum is usually a safe procedure. If the skin reaction is positive, serum administration is generally con-

traindicated unless every facility is at hand to treat a possible severe reaction.

Ophthalmic test. One drop of a 1:10 dilution of serum is dropped into the conjunctival sac. If definite congestion of the conjunctiva develops within from fifteen to twenty minutes with a sensation of itching and burning of the eye, a dangerous sensitiveness is indicated, and intravenous injection is contraindicated unless "desensitization" is practicable. Should the local reaction be marked, it may readily be controlled by prompt application of epinephrine (1:1000) to the eye.

"Desensitization." The procedure of "desensitization" and the therapeutic administration of serum are not advised in the case of patients with a positive skin or ophthalmic test except under conditions such as may be found in a well-equipped hospital. Serum therapy even under these conditions must be considered hazardous. The following procedure has been used in attempted desensitization. Subcutaneous injections of the serum, beginning with 0.01 ml. or even less, are given at one-half hour intervals until 1 ml. is reached by doubling or tripling the dose if no reaction develops. If 1 ml. injected subcutaneously incites no reaction, 0.1 ml. may be given intravenously one-half hour later. If there is no reaction, the doses may be increased very gradually until the desired amount has been administered. With a few individuals the limit of tolerance will soon be reached.

When an interval of more than three days elapses between injections of serum, the danger of serious reaction is considerable and fatal results even after attempted desensitization have been reported.

Administration of Preparations

In the administration of prophylactic and therapeutic products aseptic technic is essential. Each preparation before being released for distribution is subjected at the laboratory to rigid cultural and animal tests of sterility and harmlessness. Corresponding care should be taken by the physician at the time of injection. The directions given in the circulars accompanying the various products should be followed closely.

Preparations for injection. The skin over the selected area should be thoroughly cleansed with soap and water, then disinfected with alcohol or with tincture of iodine applied to the dry surface. Variations in procedure, when required, are indicated in the circulars accompanying the products.

The syringe should be boiled for at least five minutes immediately before use. A separate, freshly sterilized needle should be taken for each injection.

To remove material from a container through the special rubber stopper, the following procedure should be observed:

Use a sterile syringe on which the needle fits with an air-tight joint.

Wipe off the top of the rubber stopper with disinfectant.

Draw up the plunger of the syringe to the graduation corresponding to the volume to be withdrawn from the bottle.

Insert the needle straight through the center of the stopper so that the tip protrudes a short distance beyond the inner end of the stopper.

Invert the bottle and force air from the syringe into it. Avoid too great pressure.

Keeping the inverted bottle uppermost, release the pressure on the end of the plunger. If necessary, repeat the last two steps until approximately the desired volume of material flows into the syringe.

Holding the plunger firm at the desired graduation, withdraw the needle from the stopper.

Severe or Other Unusual Reactions Following Administration

Severe or other unusual reactions following the use of any of the products distributed by the State should be reported immediately to the central laboratory in Albany; also any defect in the container, unusual appearance of the material, etc. The information should always include the lot number of the preparation and the return date on the package.

Reports on the Use of Products

It is of the utmost importance for the maintenance of high standards of production that the laboratory be kept informed as to the efficacy of the preparations distributed. The various laboratory tests used in the standardization of biologic products afford important criteria regarding therapeutic values, but it is to the physician that the laboratory must turn for final proof based upon clinical experience. All the information indicated on the report forms that accompany many of the State preparations is required for the correct evaluation of results. Thus, by filling out the forms completely and returning them promptly, the clinician makes possible for himself and his patients a more efficient laboratory service. The reports on the use of the various products are all of value to the laboratory; many, utilized in publications from the Division of Laboratories and Research, have contributed materially to the progress of vaccine and serum therapy. The collaboration of many physicians in the State has already been obtained and is keenly appreciated; with further realization of the importance of these records the hearty cooperation of all physicians in supplying accurate reports is looked for.

LABORATORY AIDS IN COMMUNICABLE DISEASES

Amebiasis

Although the incidence of clinical amebiasis in the district served by this Division is low, subclinical infection with *Endamoeba histolytica* may be more widespread than is commonly realized. Amebiasis is a disease of the tropics, but by no means all of the patients suffering from it give a history of having lived in tropical climates. The condition is much more prevalent among inmates of institutions for the insane and mental defectives, owing to the habits of this type of patient, than among the general population.

Specimens for Laboratory Examination

Examinations for *End. histolytica* should be made in a local laboratory. The specimen should be passed into a warm container and should be submitted immediately for examination. If the patient is ambulatory, he should go to the laboratory for the collection of specimens, since motile forms tend to lose their characteristic appearance shortly after the stool has been passed. Cysts, on the other hand, may retain their distinguishing characteristics for several days. Oily medication renders the specimen unfit for examination. If the stool is formed or semiformed, a saline purge may be necessary. Examination of from six to ten specimens may be required unless a purge has been given, in which case three stools should be sufficient. Every patient with symptoms suggestive of dysentery should receive a sigmoidoscopic examination when the inciting agent cannot be demonstrated in the stools.

A bacteriologic examination of the feces should be made, since in some instances bacillary incitants of enteric disease will be found, as well as amebae, or the case may be one of bacillary rather than amebic dystentery.

Anthrax

Infections with the anthrax bacillus are usually incurred by handling certain animal products such as hides, hair, or wool, especially those that are imported. Before their manufacture and sale were prohibited in New York State (Sanitary Code, Chap. IX, Reg. 4), shaving brushes containing horsehair represented a particular hazard. Infection usually takes place through the abraded skin and is followed by the formation of a charactertistic pustule, but the microorganisms may gain entrance through the alimentary or

the respiratory tract. The primary lesion usually occurs within from twelve to twenty-four hours after infection. Diagnosis must be based on the history, clinical manifestations, and the finding of large Gram-positive bacilli in films prepared from the pustule. Prompt laboratory examination is essential, since the nature of the infection should be determined as soon as possible. For purposes of confirmation, the anthrax bacillus should be isolated and its identity proved by cultural and animal tests. Since the spores of *Bacillus anthracis* are highly resistant, all contaminated material should either be burned, or boiled in 10-per-cent cresol for one hour, or sterilized in an autoclave.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, the following material should be submitted for examination to a laboratory approved for the purpose: (1) exudate from the lesion on a sterile swab (tube outfit with swab); (2) films of the exudate on glass slides (slide outfit).

If microorganisms having the morphology of *B. anthracis* are found in films from the lesion, a preliminary report can be made at once. Cultural and animal tests, which may be somewhat time-consuming, are required for complete identification of the anthrax bacillus.

Blood Plasma

Dried blood plasma declared surplus to the needs of the Army and Navy was made available early in 1946 to the State Department of Health by the American Red Cross for use in civilian medical practice. The material has been sent directly to most of the hospitals in the State registered with the American Medical Association and to district laboratory supply stations. Under the terms of agreement with the Red Cross, the plasma must be supplied without any charge to the physician, the hospital, or the patient. Hospitals, however, may make a moderate charge to cover its administration. A request form must be made out by the physician or hospital for each patient for whom plasma is furnished. A report form is attached to each unit of plasma and it is of the utmost importance that it be completed and returned to the Central Laboratory. The lot number should always be given; if a serious reaction occurs the physician should notify the district state health officer or this Division immediately. These reports are essential as a basis for improvements in the service. The hazard of serum jaundice (hepatitis) due to infected lots of

Red Cross plasma should restrict the use of dried blood plasma to emergencies in which no substitute is available.

Chancroid

The inciting agent in chancroid, *Hemophilus ducreyi,* can sometimes be demonstrated in films prepared from fresh exudate from the lesion, and stained with special stains designed to demonstrate the characteristic arrangement of the microorganisms. The incitant can be isolated only when the condition of the lesion is favorable, and a specially prepared culture medium can be inoculated promptly after collection of the exudate. Failure to demonstrate the presence of *H. ducreyi* does not necessarily mean that the patient does not have chancroid; the diagnosis often must be made on the clinical findings alone. Thus, laboratory examinations have been required only for evidence of syphilis.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, the specimens specified under Syphilis, p. 81, should be submitted to an approved laboratory for examination, in order to detect concurrent syphilitic infection.

Product Supplied by the Laboratory

Sulfadiazine in bottles of forty 0.5-gram tablets is distributed for treatment through most of the district laboratory supply stations.

Cholera, Asiatic

The inciting microorganism, *Vibrio cholerae,* is found in the rice-water intestinal discharge from infected persons and also in the vomitus, and can be isolated from the feces of convalescents and carriers. It gains entrance to the body through the ingestion of contaminated food and water. The microorganisms usually disappear from the stools within three or four days. They may be found, however, for from seven to ten days, occasionally for as long as two weeks, and more rarely for three or four months. During an epidemic, the carrier rate may be from twenty to thirty per cent.

Federal quarantine regulations at ports of entry have been highly effective in excluding cholera. Modern methods of transportation, however, may introduce a new hazard from tropical diseases.

If a diagnosis of cholera is considered, the district state health officer should be notified at once by telephone.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, the following material should be submitted for examination to a laboratory approved for the purpose: (1) a specimen of feces in a sterile container without preservative (jar outfit); (2) 10 ml. of blood to be examined for evidence of typhoid fever (typhoid tube outfit). Also, a specimen of feces in 30-per-cent buffered glycerol (typhoid jar outfit) should be submitted to be examined for other bacterial incitants of enteric disease. If possible, the specimens should be delivered to a laboratory by messenger.

The presence of actively motile, Gram-negative, comma-shaped spirilla can be determined promptly by microscopic examination of specimens of feces. The identification of *V. cholerae* requires data concerning morphologic, cultural, and serologic properties.

Diarrhea

Outbreaks of diarrhea are of frequent occurrence, particularly during the summer and autumn. Except in cases of acute enteritis incited by microorganisms of the salmonella group or by dysentery bacilli, comparatively little is known regarding the etiology of such outbreaks. Even when investigations are undertaken promptly, bacilli usually associated with enteric disease often cannot be demonstrated. The role of certain groups of microorganisms such as the paracolon group in the etiology of diarrheal diseases has yet to be determined. An outbreak recently investigated by the Division proved to be due to a filtrable agent or virus which reproduced the disease in human volunteers who ingested infective material. The virus has not been established in experimental animals, and laboratory diagnosis is impractical at this time. The incidence of diseases of this type depends primarily on sanitation and methods of handling food.

Recent studies in the Division of Laboratories and Research have indicated that epidemic diarrhea may be due to a filtrable agent, presumably a virus, present in the feces and pharyngeal washings. In the epidemic studied, bacteria-free filtrates were capable of inducing the disease in volunteers. Whether epidemic diarrhea of

the newborn is also due to such an agent is unknown but is suspected. Practical laboratory tests have not been developed. In the presence of an epidemic an effort should be made to isolate a bacterial incitant. In case the causative agent is not determined, the Central Laboratory should be consulted. Outbreaks of diarrhea among the newborn should be reported to the district state health officer at once, without waiting for the results of laboratory findings.

Specimens for Laboratory Examination

Specimens of feces may be submitted (typhoid jar outfit containing 30-per-cent buffered glycerol). They should be supplemented by blood specimens for agglutination tests (typhoid tube outfit) collected at the time the patient is acutely ill and also from two to three weeks after recovery.

Diphtheria

Diphtheria often occurs in epidemic form. The inciting microorganism, *Corynebacterium diphtheriae,* usually becomes localized in the throat, producing characteristic lesions on the mucous membrane of the pharynx, tonsils, or larynx, sometimes extending into the trachea. Similar lesions may occur in the nose and, in rare instances, the conjunctiva, the vagina, and in wounds. The possibility of diphtheritic gangrene should be considered when lesions on the skin fail to heal.

Three classes of individuals may harbor morphologically typical diphtheria bacilli in the throat or nose: (1) those having, or convalescing from, diphtheria; (2) those who, without having contracted the disease themselves, have acquired the microorganisms through contact with others ("contact" carriers); and (3) those who give no history of either having had the disease or having been in contact with patients or carriers ("noncontact" carriers). Diphtheria antitoxin should be given without delay to every patient having clinical diphtheria, whether or not diphtheria bacilli are found, as well as to patients with sore throat when diphtheria bacilli are present.

The period of communicability lasts until virulent bacilli are no longer present in the secretions and lesions. The persistence of *C. diphtheriae* after the clinical symptoms of the disease have subsided is variable. In exceptional instances, virulent diphtheria bacilli remain in the throat or nose for nine weeks or more. The

usual length of time, however, is from one to two weeks. In over 90 per cent of all cases, the bacilli disappear within four weeks. In accordance with the Sanitary Code, if diphtheria bacilli continue to be present in cultures, the health officer in his discretion may release the patient from isolation 30 days after clinical recovery, provided the mucous membranes appear normal and there are no abnormal discharges from the nose, throat, or ears.

Carriers of C. diphtheriae. Persons who become persistent carriers of diphtheria bacilli are usually found to have some abnormal condition in the throat or nose, most often diseased tonsils. While penicillin has not been found effective in treatment of diphtheria, reports have indicated that prolonged local application of large doses may terminate the carrier condition.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, a culture from the throat on Loeffler's blood-serum medium, and, if symptoms of rhinitis are observed, a culture from the nose also (diphtheria culture outfit) should be submitted for examination to a laboratory approved for the purpose. When a virulence test is desired, it should be requested on the history form. When a diphtheritic infection in a wound or on the skin is suspected, a culture should be examined.

In the preparation of cultures for laboratory examination, the following directions should be observed: only Loeffler's medium that is in a satisfactory condition should be selected; a culture should not be taken immediately after the patient has used an antiseptic as a spray or gargle; the inoculum should be collected at the margin of, or from beneath the membrane rather than from the surface; separate specimens should be submitted from the nose and throat; the medium should be inoculated by rubbing the entire surface lightly and thoroughly with the swab without breaking the surface of the medium or pushing the swab into it.

A frequent source of annoyance is the contamination of medium with microorganisms that overgrow the culture and make a satisfactory examination impossible. Often, this condition results from the presence of a microorganism in the nose or throat that produces a slimy growth on the culture medium. Irrigation of the nose and throat with warm sterile physiologic salt solution may remove the exudate containing such bacteria.

Cultures are incubated at from 35° to 37° C. for from eighteen to twenty-four hours. Morphologic examinations are then made for diphtheria bacilli. When a virulence test is undertaken, the microorganism is obtained in pure culture, its morphology and cultural characters are studied, and its virulence is tested.

When sufficiently large numbers of morphologically typical diphtheria bacilli are present, a suspension of the culture may be tested by the intracutaneous method, thus making an early report possible in case virulent diphtheria bacilli are demonstrated.

Products Supplied by the Laboratory

In addition to the laboratory aids in diagnosis, there are available to the physician in the control of diphtheria, laboratory preparations for determining susceptibility, for inducing active or passive immunization, and for effecting cure. They are distributed through the district laboratory supply stations.

Outfits for the intracutaneous test of susceptibility to diphtheria toxin (Schick). The intracutaneous test of susceptibility to diphtheria toxin (Schick) consists of the injection into the skin of 1/50 of the minimum dose of diphtheria toxin fatal to a guinea pig. When there is insufficient antitoxin in the tissues to neutralize the toxin, a local reddening is induced at the site of injection; when sufficient antitoxin is present, there is no toxic reaction. The test, therefore, is designed to differentiate those who have from those who have not sufficient antitoxin in their blood to render them immune to diphtheria. Reliable results are dependent upon great accuracy in procedure, and the correct interpretation of the reactions requires considerable experience. A control injection of heated toxin dilution should always be made since some persons react to the protein in the material, especially after immunization.

The percentage of individuals susceptible to diphtheria at different ages varies in different localities and under different conditions. In general, the rural population is more susceptible than that of crowded cities. Adults are less susceptible than children. When practicable, the test should be performed before active or passive immunization against diphtheria is undertaken, especially in the case of older children and adults. A retest is necessary after active immunization to determine definitely whether immunity has developed.

In the outfits for the test are two bottles, one containing diluted diphtheria toxin for the test dose, the other containing diluted heated toxin for the control injection. The toxin in each bottle is

so diluted with buffered salt solution that the required amount for the test is contained in 0.1 ml. The toxin in the control dilution has been heated to destroy its toxicity and is used to determine sensitivity to the protein present. The outfits are distributed in two sizes; one in which the bottles contain 5 ml., or sufficient for from twenty to forty tests, and the other, 2 ml., or sufficient for about ten tests.

Since unfavorable temperature and exposure to air or light may cause deterioration of the toxin, the contents of individual containers should be used only for tests made at one time. When many tests are to be performed, removal of the stopper and the use of a long, sterile needle (gauge 15, 2 inches) to fill the syringe has been found convenient. Any water remaining in the needle should first be emptied and the syringe and needle rinsed with a small amount of the material for the test. When outfits are requested, the number of persons to be tested should be given, and if the work is to be done in different groups or on different days the number of persons in each group to be tested should also be stated.

The test. One-tenth milliliter of the toxin dilution is injected intracutaneously on the flexor surface of the left forearm and a similar volume of the heated toxin dilution on the right forearm. Special syringe outfits with a separate syringe for the control test can be purchased. Syringes and needles that have been previously employed for tuberculin tests should not be used. A freshly sterilized needle for each child is recommended. When only one reading is practicable, it should be made on the fifth, sixth, or seventh day after the injection. It has been found convenient to make readings and give the initial immunizing dose to those showing positive reactions, on the seventh day.

Detailed directions accompany each outfit for the intracutaneous test. To secure dependable results it is essential that they be followed closely.

Diphtheria Toxoid

Active immunization. Since the incidence of diphtheria is highest in young children and the mortality greatest in those under five years of age, the active immunization of children of pre-school and school age is an important preventive measure. The immunity usually derived by infants from their mothers is lost during the first months after birth, so that it is desirable that the immunizing injections be

given at the age of six months or shortly after. The fact that in children under six years slight, if any, reactions are induced is an added advantage of early administration.

> Most children are susceptible to diphtheria, hence the preliminary test of susceptibility (Schick) of those under ten years of age is often omitted. Since fewer adults are susceptible to diphtheria, and more react to the immunizing doses of toxoid, the preliminary test should always be made to determine the need for immunization. In order to ascertain whether immunity has developed, an intracutaneous test should be made from four to six months after the last immunizing injection. Protection against diphtheria cannot be assumed without a negative test. In the case of children who received toxoid before two years of age, an additional injection of 1.0 ml. as a further stimulus should be given when the child reaches school age or before. A retest to determine susceptibility may first be made.

Diphtheria toxoid, precipitated and unprecipitated, and diphtheria-tetanus toxoid, precipitated, are prepared, tested, and distributed by the Division of Laboratories and Research for active immunization against diphtheria.

Diphtheria toxoid, precipitated, contains no horse or other serum. It is prepared by subjecting potent diphtheria toxin to the action of formalin and heat until it has become detoxified. The active principle is then adsorbed by suitable chemical reagents and the resulting precipitate washed and resuspended in physiologic salt solution. The presence of the precipitate retards absorption and is considered an important factor in the favorable results reported from the use of this purified stable preparation. The response to two doses of this material is superior to that following three doses of the unprecipitated preparation, both in initial response and in persistence of immunity; disturbing reactions to either the initial doses or the stimulating dose in persons up to 15 or even 18 years of age are unusual. The precipitated material is also recommended for use as a stimulating dose.

Precipitated toxoid is distributed in bottles containing 10 ml. and in smaller bottles containing sufficient material for two injections. Requests for the material should always state the number of children to be immunized.

Administration. In order to ensure correct dosage, it is essential that equal amounts of the suspension be injected. The bottle should be thoroughly shaken just before it is opened and rotated each time the syringe is filled. The syringe should be rotated similarly before each injection. Two injections of 1.0 ml. each a month apart are

recommended and a stimulating dose of 1.0 ml. prior to entering school. For additional stimulating doses, a reduced amount, even as small as 0.1 ml., will probably be satisfactory. Special care should be taken to ensure the injection of the full amount and to prevent loss by oozing. The injection should be made subcutaneously on the outer side of the upper arm, the left for the first and the right for the second if given. Deep or intramuscular injections should be avoided.

Diphtheria toxoid, unprecipitated, contains no horse or other serum. A supply of this material is maintained at the central laboratory for special use, mainly for the immunization of those who are sensitive to diphtheria protein and of the older age groups. There is little evidence, however, that precipitated toxoid causes more severe reactions than the unprecipitated. In requesting material, physicians should indicate the reasons for preferring unprecipitated toxoid.

Diphtheria toxoid, unprecipitated, is distributed in bottles containing 5 ml. and in smaller bottles containing sufficient material for the complete immunization of one person or for one immunizing injection of three persons.

Administration. Injections are given subcutaneously, alternately on the outer side of the upper arm, beginning with the left arm. Three doses of toxoid of 0.5 ml. each at 2- or preferably 3-week intervals are recommended. On account of the small volume, special care should be taken to inject the full amount and to prevent loss by oozing. For persons over 15 years, a modified dosage is advised. If the control for the intracutaneous test of susceptibility indicates a high protein sensitivity, the size of the doses may be reduced to 0.2, 0.4, and 0.4 ml. at 2- or preferably 3-week intervals. It may be advisable to reduce the doses still further and give a fourth dose.

Diphtheria-tetanus toxoid, precipitated, combines the advantages of both preparations in a volume dose approximating that of either and induces no more reactions. It contains no horse or other serum. The material is prepared by mixing equal parts of each precipitated toxoid. It is considered an effective prophylactic agent for the active immunization of children against diphtheria and tetanus.

Diphtheria-tetanus toxoid, precipitated, is distributed in 10 ml. and 2.5 ml. amounts. Requests for the material should always state the number of persons to be immunized.

Administration. The same precautions as for precipitated diphtheria toxoid should be observed. Two subcutaneous injections of 1.0 ml. each a month apart are required. In general, immunity to diphtheria develops following two injections of the toxoid, but it cannot be assumed without a negative intracutaneous test. No intracutaneous test is available, however, to determine whether immunity to tetanus has been acquired. In order to maintain an adequate level of immunity, therefore, a stimulating dose of precipitated diphtheria-tetanus toxoid or of tetanus toxoid alone should be administered at the end of a year. For children immunized when less than one year old, an injection of the combined toxoid at this time will serve as the recommended stimulating dose at two years for immunization against diphtheria as well as tetanus.

A stimulating dose of toxoid at the time of an injury for which ordinarily a prophylactic injection of tetanus antitoxin would be given, is considered to be sufficient to protect against tetanus infection. In case of any doubt as to previous active immunization with tetanus toxoid, a prophylactic dose of tetanus antitoxin should be administered.

Diphtheria Antitoxin

Passive immunization. Protection of contacts previously shown by the intracutaneous test to be susceptible to diphtheria and of those whose susceptibility is not known may be affected by passive immunization with diphtheria antitoxin. Concentrated, purified diphtheria antitoxin produced by the State laboratory is distributed through supply stations in packages of 1,000 units for prophylactic use. The dose for adults is 1,000 units given subcutaneously; for children, from 500 to 1,000 units, depending upon body weight. Such persons will be protected for a period usually of about two weeks.

Curative treatment. When the lesion in the throat is typical, and especially when in suspected laryngeal diphtheria croupous symptoms develop, antitoxin should be administered immediately and a culture taken for bacteriologic diagnosis. The harmful effects in diphtheria are due to the diphtheria toxin which diffuses through the body from the local lesion in the throat. Toxin that has become united with the cell substance is probably not affected by antitoxin. When sufficient toxin has combined with the body tissues to cause death, no amount of antitoxin will bring about recovery. Hence, it

is of the utmost importance to begin treatment as early as possible in the course of the disease.

An early and liberal single injection is always preferable to smaller divided doses. The initial dose should be sufficient, but if the clinical symptoms persist the dose must be repeated, possibly increased. The antitoxin for therapeutic use is distributed in packages containing 5,000 and 10,000 units. Directions are contained in each package.

Administration. Immediate curative action is best secured by intravenous injection which is from three to four times as effective as injection into the subcutaneous tissue, but only physicians experienced in intravenous serum administration should practice it. In late or in severe cases, as in emergencies of laryngeal diphtheria, it is much to be preferred. Intramuscular injection, because of more rapid absorption, is more effective than subcutaneous.

Initial Dosage in Diphtheria

	Child (under 60 lbs.)	Adult	
Mild	3,000-5,000	3,000-5,000	Intramuscular or Subcutaneous
Moderate	5,000-10,000	5,000-15,000	Intramuscular
Severe to Malignant	10,000-25,000	20,000-50,000	Intramuscular or Intravenous

The smaller dose is adequate for intravenous injection.

Intravenous injection is made into the median basilic vein, the antitoxin preferably being diluted with warm sterile physiologic salt solution. In infants, if necessary, the external jugular vein may be used. For precautions against anaphylactic reactions, see page 19.

Dysentery, Bacillary

At least four distinct types of Shigella (dysentery bacilli) have been recognized as incitants of bacillary dysentery—Shiga, Schmitz, Sonne, and Flexner. Infections with the last three occur relatively infrequently in New York State except for occasional outbreaks among inmates of institutions; infections with the Shiga type, in which the mortality is relatively high, are extremely rare. Several subtypes of the Flexner type may be differentiated by agglutination tests in specific sera. These tests are not recommended as a routine procedure, however, since the results are seldom of more than academic interest.

The dysentery bacillus usually enters the body by the mouth, although infection may result from the use of unsterile tubing or

other instruments employed in the administration of enemas or in similar procedures.

The microorganism is found in the feces, but seldom if ever in the blood stream or in the urine. When a person has recovered from bacillary dysentery, the incitant can usually be isolated from the stools for only a relatively short time. Occasionally, however, patients develop symptoms of colitis and remain carriers of dysentery bacilli for long periods. The results of serologic tests for evidence of bacillary dysentery have proved disappointing. Blood from many normal individuals has been found to agglutinate dysentery bacilli, while, on the other hand, the titer of serum from patients with dysentery, at least when the specimens are collected early in the course of the disease, may be no higher than that of persons who are not ill. Consequently, blood from patients with symptoms of dysentery is usually examined for evidence of typhoid fever, since the clinical manifestations in these enteric infections may sometimes be similar.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, the following material should be submitted for examination to a laboratory approved for the purpose: (1) a specimen of feces (typhoid jar outfit containing 30-per-cent buffered glycerol); and (2) 10 ml. of blood to be examined for evidence of typhoid fever (typhoid tube outfit).

The intestinal discharge consisting of blood and mucous as obtained in early stages of the disease, especially when relatively free from fecal matter, is the most suitable for examination.

Encephalitides (Virus)

Human encephalitis may result from infection with one of a large number of neurotropic viruses, and other viruses which are not ordinarily neurotropic may occasionally cause encephalitis. In the United States the primary encephalitides of greatest practical significance are St. Louis, Western equine, and Eastern equine encephalitis. They are transmitted by blood-sucking arthropods from a large natural reservoir of infection, more dense in certain geographical areas than others. Birds and barnyard fowl as well as horses constitute the most important natural reservoirs. The diseases occur predominantly during the summer or, more precisely, during the mosquito season.

Polioencephalitis, rabies encephalitis, and postvaccinial or postinfectious demyelinating encephalitis also are widely prevalent and require differentiation from other types. The etiology of encephalitis lethargica, the incidence of which has declined markedly in the United States since 1926, is unknown. Mumps meningoencephalitis, lymphocytic choriomeningitis, tuberculous meningitis, syphilitic meningitis, or brain abscess may be the cause of lymphocytic reactions and other abnormalities in the cerebrospinal fluid and frequently must be considered in the differential diagnosis.

Specimens for Laboratory Examination

The results of a cell count, protein determination, colloidal gold test, complement-fixation test for syphilis, and bacteriologic examination of specimens of cerebrospinal fluid are useful in evaluating the clinical manifestations. See Syphilis, p. 81. Etiologic diagnosis requires either the isolation and identification of the causative virus or serologic evidence of specific virus infection.

During early acute illness these viruses are most frequently found in the blood, although they have been isolated from the cerebrospinal fluid in rare instances. It is desirable that both cerebrospinal fluid and heparinized blood be submitted to the laboratory. The specimens should be frozen immediately after collection and shipped in dry ice.

An early acute-phase and two convalescent-phase specimens of serum should be examined. The results of serologic tests may be helpful. Three specimens of blood should be submitted for these examinations—one collected during the early acute phase, and two during the convalescent phase. One of these latter should be collected from 2 to 4 weeks, and the other from 6 to 12 weeks after onset of illness.

Food Poisoning

The term "food poisoning" is usually employed to designate any illness following the ingestion of food containing certain toxigenic microorganisms or their products.

Botulism. The most serious, but in this country the least common, form of food poisoning is that known as botulism, which results from ingestion of food containing toxin produced by *Clostridium botulinum*. Botulinus toxin is highly poisonous and is not destroyed in the stomach or intestine. Several types of the botulinus

bacillus have been recognized, but those most commonly associated with botulism in man are types A and B. Strains of type E have been isolated in New York State from canned and also from smoked fish. The products, which had been imported, had not been sterilized by heat. In both instances, individuals who had eaten the fish developed symptoms of botulism; there were two deaths.

The source of infection in North America has, in most instances, proved to be canned nonacid fruits and vegetables, while in Europe, contaminated ham, sausage, and fish have figured most prominently. Individuals engaged in home canning should be aware of the dangers of using improper methods. Heating in a pressure cooker is the most satisfactory way of destroying the spores of *Cl. botulinum.* In the case of foods that are definitely acid such as most fruits, tomatoes, pickled beets, ripe pimentos, and rhubarb, the botulinus spores may be destroyed within a reasonable time at the temperature of boiling water. Provided nothing is added to reduce or counteract the acidity of these foods, processing in a pressure cooker is not necessary. The bland nonacid foods, however—including all vegetables other than those mentioned above, such as asparagus, peas, beans, corn, beets, and greens, and also meats and poultry—can be rendered safe with speed and surety only at the high temperature obtainable in a reliable steam pressure canner. Physical evidence of spoilage may or may not be present in food contaminated with *Cl. botulinum.* Fatal cases have resulted from the tasting of food in which signs of decomposition were scarcely noticeable and were unaccompanied by any unpleasant taste or odor. Botulinus toxin is destroyed by boiling, but the heating of the food must be sufficient to insure raising every portion to the boiling point and continuing the heating for an additional fifteen minutes.

In botulism, the disease process results from the affinity of the toxin for the nervous system, and death occurs as a result of respiratory paralysis. In characteristic cases, the symptoms include: initial transitory gastrointestinal symptoms followed by symptoms of a rapidly progressive paralytic neurologic syndrome: dilated nonreactive pupils, diplopia, difficulty in swallowing, obstinate constipation, distention and eventually respiratory paralysis. The clinical picture closely resembles that following atropin poisoning and bulbar paralysis. The period of incubation is variable (less than twenty-four hours or as long as a week), and only relatively mild gastrointestinal symptoms (vomiting once or twice and constipation) may precede the development of the characteristic paralysis.

Other types of food poisoning. Food poisoning of the more common type can usually be attributed to toxic products of certain common bacterial species, principally staphylococci. The illness is generally characterized by a short incubation period, sudden onset with abdominal pain, nausea and vomiting, and offensive diarrhea. Fever is low-grade; the temperature rarely exceeds 102° F. or 103° F. In severe cases, there may be faintness, muscular weakness, and shock. *Salmonella* and dysentery bacilli may be conveyed by food, and infections with these species are, therefore, sometimes designated as food poisoning.

Staphylococci and other types of bacteria that produce toxic substances in food may be derived from lesions on the hands or from the nose of the food handler, or from raw milk from cows with diseased udders. Certain foods, especially cooked meat with gravy or cream sauce and custard-filled pastries, offer an excellent medium for the development of this type of bacterium, but determination of the incitant and the source of contamination is often difficult. Bakers and other distributors of cooked foods, and the general public, should realize the importance of storage at temperatures unfavorable to bacterial growth. In all instances, the toxic food stuffs have been found to have been kept, for a few hours at least, at a temperature that favors the development of bacteria.

Since rodents and fowls are known to harbor many species of *Salmonella,* contamination of food with their feces is probably one of the most common means of disseminating these microorganisms. They may be found also on the shells or in the contents of eggs. Human carriers or cases may be the source of *Salmonella* and also of dysentery bacilli. The source of *Cl. botulinum* is usually the soil.

Specimens for Laboratory Examination

After an investigation has been made to determine the articles of food consumed by all who are ill, the suspected material should be sent to an approved laboratory, together with the pertinent data, including clinical manifestations of the illness. If canned, a portion of the food taken from the same container or lot as that eaten by the patient should be collected. In the case of botulism, even washings from the can may be adequate for testing. Since the symptoms in botulism may appear at any time from within twenty-four hours to several days after ingestion of the food containing the toxin, any food eaten within that period should be considered.

Food to be examined for *Staphylococcus aureus* or other microorganisms producing enterotoxins is satisfactory only when it has been refrigerated to prevent bacterial growth subsequent to the time when portions of it were consumed. Strains of *Staph. aureus* that produce enterotoxins, and those that do not, cannot always be differentiated with certainty.

Specimens from the patients should also be submitted: 20 ml. of blood to be studied for the presence of the botulinus toxin, a specimen of feces without preservative to be examined for *Cl. botulinum* (miscellaneous jar outfit), and another specimen of feces in 30-per-cent buffered glycerol (typhoid jar outfit) to be examined for *Salmonella* and other bacillary incitants of enteric disease. *Cl. botulinum* and its toxic products, however, can be more readily demonstrated in food than in these types of material.

Products Supplied by the Laboratory

Botulinus antitoxic sera. Serum therapy in cases of human botulism has not been used sufficiently to warrant a definite statement as to its practical value; that is, how early the serum must be given to be effective or how late in the course of the disease its injection becomes useless. Experiments with animals indicate that the serum may be of value if given very promptly after the first symptoms are noted or even before symptoms if it is known that an individual has consumed food containing the toxin. Two types of *Cl. botulinum,* designated as A and B, are most frequently associated with human cases. A few cases due to type E have been reported in New York State, but for this type no serum is now available. The types produce different toxins. Since the immediate determination of the type is not practicable, a multivalent serum, or both types of univalent sera, A and B should be given. The two univalent sera may be combined or given separately. Univalent botulinus antitoxic sera of types A and B are produced by the State laboratory and distributed in packages containing 20 ml. for immediate use. The type A serum is of exceptionally high titer and unconcentrated; the type B serum is concentrated.

Requests for the sera should be sent by telephone or telegram at the earliest possible moment after botulism is suspected to the Central Laboratory, Albany, or the Branch Laboratory, 339 East 25th Street, New York City 10, or to one of the following where a limited supply is maintained for immediate emergency use: the departments

of health at Buffalo, Syracuse, and Rochester, the Binghamton City Hospital, the district state health officer at Gouverneur.

Administration. An initial dose of from 60 to 120 ml. (two bottles of type B for each one of type A) should be given intravenously by gravity at the earliest possible moment. The dose may be repeated in from 6 to 8 hours unless it is apparent that no beneficial result can be expected from further serum administration.

A prophylactic dose of 10 ml. of antitoxin, multivalent or combined, given intramuscularly, has been recommended for persons who may have consumed some of the suspected food but have not yet developed symptoms. The appearance of any suggestive symptoms should be followed by the administration of the full dose intravenously. A circular giving detailed directions and a report form are contained in each package. For precautions against anaphylactic reactions, see page 19.

Gas Gangrene

Infection with gas-forming anaerobic microorganisms, designated as gas gangrene, may develop in any dirty wound, especially when there has been extensive destruction of tissue, as in the case of compound fractures, crushing injuries, and gunshot wounds.

The laboratory can be of little assistance in diagnosis, since the results of bacteriologic examinations are not available soon enough to be of value as a guide in treatment. Examination of a piece of traumatized muscle from the deeper portions of the wound, however, may reveal the presence of gas-forming anaerobic bacilli. Therapy to be effective must be undertaken promptly on the basis of clinical and x-ray findings.

Product Supplied by the Laboratory

Gas gangrene antitoxin. Clinical reports received have indicated the value of serum therapy in the treatment of cases of gas gangrene due to the presence in the wound of one or more species of anaerobic bacteria. Treatment should be commenced promptly. Further experience with the use of sulfonamide drugs has determined the value of these agents in the treatment of gas gangrene, either alone or in combination with serum and surgery and penicillin.

A small supply of gas gangrene antitoxin is purchased and held for emergency use at the State laboratory, Albany, the Binghamton City Hospital, the Kingston City Laboratory, and the Erie County

Laboratory, Buffalo. Requests should be made by telephone or telegraph to the central laboratory, Albany, the Branch Laboratory, 339 East 25th Street, New York City, or to the nearest station. The antitoxin, which is multivalent, is produced against the toxins of *Cl. welchii* (*B. perfringens*) and certain other species of pathogenic anaerobes. If the patient is able to pay for the material or the case is covered by compensation, it is expected that the amount supplied will be replaced promptly. The material is not furnished for prophylactic use.

Administration. An initial injection of the contents of one bottle up to that of four bottles is recommended to overcome as far as possible the general toxemia. Subsequent injections of at least the minimum dose (one bottle) may be given at from 4 to 6 hour intervals or longer depending upon the condition of the patient. Intravenous injection is advised until signs of definite improvement are noted; subsequent doses may be given subcutaneously. Detailed directions and a report form accompany each package. For precautions against anaphylactic reactions, see page 19.

If a prophylactic injection of tetanus antitoxin has not already been given, from 1,500 to 3,000 units should be administered at once and the dose repeated if the wound continues favorable for the development of tetanus infection.

Glanders

Glanders, primarily a disease of horses, can be transmitted to man. The incitant, *Bacterium mallei,* is found in the nasal secretions, pus from nodules, blood, and at times in the urine, saliva, and milk. In man, the mode of infection is usually through an abrasion of the skin, but may be through the mucosa of the mouth and nose. A nodule appears at the site of infection, accompanied by lymphangitis and swelling. A general pustular eruption may occur. While the disease is usually acquired from contact with horses, it may be transmitted from man to man.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, the following material should be submitted for examination to a laboratory approved for the purpose: (1) 10 ml. of blood (typhoid tube outfit); (2) a specimen of discharge on a sterile swab (tube outfit with swab); (3) films of discharge on glass slides (slide outfit).

So few cases of glanders occur in man that little opportunity has been afforded to evaluate the results of serologic tests. Thus, adequate material for cultural examination is desirable.

Gonorrhea

Laboratory aids in the diagnosis of gonorrhea have proved especially helpful. In acute or active cases of gonorrhea, the incitant, *Neisseria gonorrhoeae,* is nearly always demonstrated microscopically in large numbers in the discharge, and can usually be isolated if cultural examination is undertaken promptly after collection of the specimen. Cultural examinations are now seldom justified, however, since the treatment of gonorrhea with penicillin is simple and highly effective; they are no longer performed routinely at the Central Laboratory.

The results of serologic tests proved of value in some instances and, with further development, might well have become more generally useful. The results obtained with the complement-fixation test with gonococcus antigens, however, did not appear of sufficient value as an aid in the diagnosis or management of gonococcus infections to warrant continuing its use in the Central Laboratory.

Specimens for Laboratory Examination

In the male, urine, prostatic secretion, or exudate from the urethra should be submitted for examination; and in the female, exudate from the urethra, cervix, and Bartholin's glands, or, in vulvovaginitis, from the vagina. In extragenital infections, appropriate specimens such as blood, joint fluid, or cerebrospinal fluid should be submitted. Whenever the gonococcus is suspected as the incitant of a conjunctivitis, specimens should be submitted as described under Ophthalmia Neonatorium, p. 59.

Methods for Collecting Specimens

From females.—When specimens are being taken for culture, lubricant should not be used on gloves or instruments.

Urethra: If no urethral discharge is evident, the urethra should be compressed with the intra-vaginal finger and stripped toward the meatus so as to express any pus that may be in Skene's glands.

Cervix uteri: The outer surface of the cervix should be cleaned with sterile gauze or cotton and the mucous plug removed with a

cotton applicator. Then any accumulation of exudate in the posterior fornix should be removed with dry cotton. The entire cervix should be subjected to firm pressure between the blades of the speculum or by means of long-handled dressing forceps. The applicator should be inserted into the external os and rubbed with slight pressure over the mucosa lining the canal.

Bartholin's glands: If Bartholin's glands are infected, a specimen may be collected by gently massaging the glands between the thumb and intra-vaginal index finger.

Vagina: In the case of children with vulvovaginitis, specimens from the vagina should be collected with a cotton swab or a glass catheter.

From males.—*Urethra:* If no urethral discharge is evident, the prostate should be carefully massaged and the urethra compressed with the finger and stripped toward the meatus. If no exudate is obtained in this way, the patient should be asked to urinate and the first half ounce (10-15 ml.) of urine should be collected in a clean tube. This should be taken to a laboratory for microscopic and cultural examination within six hours.

Submission of Specimens to the Laboratory

For microscopic examination. Thin films should be prepared on glass slides by rolling swabs on which specimens have been collected over the surfaces of the slides or by using the edge of another slide as a spreader. The films should be thin and evenly spread. The films should be allowed to dry thoroughly in the air without heating before replacing the slides in the box, specimen sides together.

For cultural examination. If special circumstances warrant cultural examination, this should be undertaken, if possible, in a nearby approved laboratory. Otherwise, it will be necessary to obtain special outfits in which specimens can be sent through the mail successfully.

Products Supplied by the Laboratory

Procaine penicillin in syringes containing 300,000 units is distributed through certain health officers and district laboratory supply stations for use in the treatment of drug-resistant cases of gonorrhea. Sulfadiazine in bottles of forty 0.5-gram tablets is distributed to physicians and clinics through most of the district laboratory supply stations.

Granuloma Inguinale

Experience during the past few years has shown that granuloma inguinale is fairly prevalent in this country, especially among negroes. Reports indicate that while early treatment of the infection may be successful, management of chronic cases is much less promising. Donovan bodies are present as inclusions in some of the large tissue cells in the lesions. The local laboratory is in an advantageous position to assist in the diagnosis.

Specimens for Laboratory Examination

Films prepared from macerated tissue from the edge of the lesion, after the superficial exudate and crusts have been removed, should be examined for Donovan bodies. If there is doubt whether a lesion is a syphilitic chancre or one of granuloma inguinale, the films to be examined for Donovan bodies should be made before the fluid from the lesion is collected for examination for *Treponema pallidum,* since, when the lesion is swabbed with gauze as is done in the latter case, a large part of the cells containing the Donovan bodies in their most readily recognized form may be removed.

Helminthiasis

The ova and sometimes the larvae or adult forms of most species of parasitic worms (helminthes) can be demonstrated in the feces. The ova of pinworms, *Enterobius (Oxyuris) vermicularis,* can most easily be found on the skin around the anus.

Fortunately, the prevalence of diseases resulting from infections with helminthes does not seem to have increased materially since World War II. They must be considered, however, especially when there is a history of military service in districts where they are endemic.

Trematodes

Lung flukes. Infections with lung flukes occur in the Far East, chiefly Korea. Persons with chronic cough and bloody sputum who have lived in an area where the worm is endemic may be infected with these parasites (*Paragonimus westermani*). Hence, in addition to examinations for evidence of tuberculosis, a drop of bloody sputum

can be examined microscopically for the ova of this fluke. Concentration of the sputum is helpful.

Schistosomiasis. The ova of *Schistosoma mansoni* and *Schistosoma japonicum,* as well as those of the less common intestinal and biliary tract flukes, are found in the feces. In the case of *S. hematobium,* the infection is usually in the urinary tract, although occasionally, the parasite is in the liver.

Cestodes

Taenia saginata is the type of cestode most frequently found in New York State.

Nematodes

Filariasis. *Wuchereria bancrofti, Loa loa,* and *Onchocerca volvulus* are the most important filarial worms parasitic for man. *Wuchereria bancrofti* do not mature until from twelve to eighteen months after the patient has become infected. When the parasites are mature, microfilariae may be found in the blood. They can usually be found most easily in specimens taken during the night. In certain areas of the South Pacific, however, this rule does not pertain. Hence, a search may need to be made at 2- or 3-hour intervals during the entire twenty-four hours.

Loiasis. Loiasis, calabar or eye-worm disease, is prevalent in tropical West Africa. The adult worms migrate in the subcutaneous tissue of man. The microfilariae may be found in the peripheral blood or in fluid aspirated from the calabar (intermittent) swellings. Since they have been reported to be more numerous in the blood during the day than at night, specimens should be collected between 10:00 a. m. and 2:00 p. m.

Onchocerciasis. Onchocerciasis is prevalent in parts of Guatemala, Mexico, and in certain sections of Africa. Usually, the disease is characterized by small skin tumors, particularly of the scalp. The diagnosis can be made early in the disease by demonstrating microfilariae in either stained or unstained films prepared from aspirated material from the tumors. A biopsy specimen may also be examined histologically.

Trichinosis. Trichinosis is incited by *Trichinella spiralis* and results from eating raw or improperly cooked meat, usually pork, occasionally bear meat, containing the living encysted larvae of the

parasite, which are readily destroyed by thorough cooking. The larvae have been found to remain alive for several weeks in certain kinds of smoked sausages that are eaten uncooked.

The trichinella reaches the adult stage in the intestine, where breeding takes place. After from five days to three weeks, the embryos migrate to various parts of the body and, if not destroyed, become encysted within the muscle fibers. Trichinella larvae have, on occasion, been found in blood and cerebrospinal fluid, but the examination of feces is in most cases useless. Indications of infection are not usually evident, until the parasites have reached the muscles.

Blood counts furnish information of material value in diagnosis. A leucocytosis with marked eosinophilia is the characteristic finding, and repeated examination for eosinophilia is of greatest importance. Patients who have received an overwhelming infection, however, may not develop an eosinophilia. Skin tests and serologic tests are of considerable aid, but the results must be interpreted with caution.

Specimens for Laboratory Examination

One or two milliliters of fecal material without preservative (jar outfit) may be submitted for examination for most types of helminthes and their ova, or, if the presence of pinworms is suspected, a specimen collected in accordance with the following directions can be submitted:

> "A piece of 'Scotch Cellulose Tape' of half-inch width and about 8 cm. long is folded down for about a centimeter at each end, adhesive surfaces together, to form two grips for handling. In use the ribbon of cellulose is held in forceps in a loop, adhesive surface on the outer side of the loop, and is patted down on the perianal skin. The extremely adhesive surface picks up epithelial scales, fecal particles, and ova if any be present. The tape is then placed lengthwise on a microscope slide, smoothed down with the side of the forceps and examined with a 16 mm. objective like any preparation under a coverglass. If desired the folded ends, which of course do not adhere to the slide, can be trimmed off with scissors so that the tape will lie completely flat on the slide, but this is not essential." (Graham, C. F., Amer. Jour. Trop. Med., 1941, *21*, 159.)

Thick blood films should be submitted. Directions for preparation are given under Malaria, p. 49.

When trichinosis is suspected, sections of muscle in 10-per-cent formalin may be submitted to be examined for trichinellae, as well as blood films for a differential leucocyte count. In case the work

can be done in a nearby laboratory, a total leucocyte count is also desirable.

If possible, a portion of the meat or meat product thought to be the source of infection should also be submitted for examination.

Examinations for evidence of helminthiasis can best be undertaken in a local laboratory.

Infectious Hepatitis (Acute Catarrhal Jaundice) and Homologous Serum Jaundice

Infectious hepatitis is characterized by prodromal headache, malaise, and gastrointestinal disturbances followed by jaundice of variable degree. The incubation period is three to six weeks. Leucopenia with relative lymphocytosis often occurs, and is of assistance in differentiating this disease from leptospiral (spirochetal) jaundice, in which the leucocyte count is usually well over 10,000 per cu. mm. The disease varies from mild cases without jaundice to fatal acute yellow atrophy of the liver. The causative agent has not yet been transmitted to experimental animals but human volunteer studies have shown that it is filtrable, and presumably a virus. The agent is present in the blood, stool, urine, and respiratory discharges of infected persons. Clinical tests of liver function and examination for bile derivatives in blood and urine supply helpful information.

Homologous Serum Jaundice. Following blood or plasma transfusion, or inoculation with biologic products containing human serum, individuals may develop a similar disease, so-called homologous serum jaundice. The incubation period may be as long as four months. The causative agent closely resembles that of infectious hepatitis.

Infectious Mononucleosis

The clinical manifestations of infectious mononucleosis are extremely variable, and often simulate other unrelated diseases. They include fever, lymphadenitis, sore throat, and jaundice accompanied by characteristic findings in the blood. The epidemic form of the disease, which usually occurs among children, is commonly referred to as glandular fever, but this is now believed to be the same disease as infectious mononucleosis. The etiology has not been established, but the agglutination test with sheep red blood cells is an aid in diagnosis. Injections of horse serum, particularly when serum sickness occurs, and administration of bacterial vaccines or other types of

foreign protein may also give rise to agglutinative properties for sheep red blood cells.

Specimens for Laboratory Examination

Blood films (slide outfit) may be submitted for differential leucocyte counts and 10 ml. of blood (typhoid tube outfit), for serologic tests.

Influenza (Virus)

Epidemic and sporadic influenza are caused by at least two immunologically distinct types of viruses, designated influenza viruses A and B. It is not known whether pandemic influenza is due to either of these types.

The virus may induce all gradations of disease from subclinical to fatal infections and characteristically predisposes to secondary bacterial invasion especially of the respiratory tract and ears. Cultural examinations of the sputum, blood, and indicated secretions from the nasopharynx should be undertaken if bacterial complications are suspected. The leucocyte count is usually normal or low in uncomplicated influenza but elevated if secondary bacterial infection has occurred. Many of the complications are beneficially affected by antibiotics and sulfonamides, but uncomplicated influenza is not. In the selection of the therapeutic agent to treat the complication, reliable bacteriologic evidence is essential.

Prophylactic vaccines against influenza viruses A and B are effective. The value of vaccines is reduced if the prevalent strain of virus is antigenically different from that used in preparing the vaccine.

Specimens for Laboratory Examination

The virus can often be detected in pharyngeal washings obtained during the first three days of disease or from lung tissue taken at autopsy. Pharyngeal washings are collected by having the patients gargle with sterile broth and expectorate the specimen into a specimen jar. Isolation and identification of influenza virus A or B require the inoculation of animals or embryonated eggs. Specimens should be shipped in dry ice.

Complement-fixation or agglutination-inhibition (Hirst-McClelland-Hare) tests will establish the diagnosis if both acute- and convalescent-phase sera are examined. Blood should be collected during the acute-phase before the end of the first week, earlier if possible.

A definite serologic diagnosis can seldom be made in the absence of an acute-phase specimen of blood since normal individuals frequently have relatively high antibody titers against influenza virus A or B.

Jaundice

Acute infectious jaundice (Leptospirosis) or Weil's disease.

Severe types of acute infectious jaundice incited by *Leptospira icterohaemorrhagiae* and *Leptospira canicola* are endemic in certain parts of Japan and Europe, and sporadic cases have been reported in this country. Rats and dogs are carriers of the leptospirae; the microorganisms are present in the kidneys and excreted in the urine. Infection of human beings apparently results from contact with the urine of infected animals. The rare occurrence of cases of leptospiral jaundice, in spite of the wide distribution of the inciting agent, has been attributed to the fact that the microorganism is extremely sensitive to drying and sunlight. It probably survives but a short time after being excreted from the animals.

Specimens for Laboratory Examination

Specimens of blood collected asceptically during the first week of symptoms and specimens of urine collected any time after onset are satisfactory for examination for leptospira provided the study can be unde-taken promptly after collection. Ten milliliters of blood (typhoid tube outfit) may be examined for agglutinative properties for these microorganisms.

Lymphogranuloma Venereum

Lymphogranuloma venereum (lymphogranuloma inguinale) is caused by a virus closely related to the psittacosis, ornithosis, and S-F and Louisiana pneumonitis viruses. Laboratory examinations will determine whether a virus of this group is the incitant of disease, but differentiation within the group is sometimes troublesome. Taken in conjunction with clinical and epidemiologic evidence, however, the results of laboratory studies may be helpful in establishing the diagnosis of lymphogranuloma venereum. Since the disease is usually acquired through sexual intercourse and is fairly prevalent, the necessary examinations for the diagnosis of syphilis and chancroid, which may coexist, should not be omitted. The intracutaneous (Frei) test is a valuable aid in diagnosis. Sulfonamide drugs are effective.

Specimens for Laboratory Examination

The virus is present in bubo pus and in biopsy specimens from involved areas. Its isolation and identification require the inoculation of animals or embryonated eggs. Specimens should be shipped frozen in dry ice.

The complement-fixation test with lymphogranuloma venereum virus antigen will establish the diagnosis if both acute-phase and convalescent-phase sera are examined and if the results are interpreted in conjunction with the clinical course of the patient.

Products Supplied by the Laboratory

Lygranum for making the Frei test is available on request to the Central Laboratory, Albany. Sulfadiazine in bottles of forty 0.5-gram tablets is distributed for treatment through most of the district laboratory supply stations.

Malaria

Very few cases of malaria occurred in New York State in the years just prior to World War II. Since members of the armed forces have returned from assignments in the tropics, the incidence has increased considerably. The anopheline mosquito, which is the intermediate host, is found in various parts of the State. Hence, human carriers who may be living here may serve as a reservoir from which the infection may be transmitted. Blood films to be examined for malaria parasites should be prepared if possible before the initiation of chemotherapy. Occasionally, sternal punctures may be advisable.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, Sanitary Code, thick and thin films of blood on glass slides (slide outfit), preferably taken from twelve to twenty-four hours after a chill, should be examined in a laboratory approved for the purpose. Repeated films may be necessary for demonstration of the plasmodia. In fulminating cases, prompt examination is important, since delay in treatment may be fatal.

For examinations required when blood or blood derivatives are to be used for therapeutic or prophylactic purposes, see Sanitary Code, Chap. IV-A.

Directions for preparing thick films. A thick film should consist of from three to five average-sized drops of blood. These may be deposited

on an area about three-quarters of an inch in diameter (the size of a dime) and promptly combined with a needle or the corner of another slide; or the surface of the slide may be touched to a large drop of blood and the slide moved in narrow circles until a preparation of the size mentioned is obtained. The film must be fairly thick in order to have sufficient blood for adequate examination, but must not be so thick that it will peel from the slide during staining. When the thick blood film is still wet, ordinary printing can just be read through it.

In order to protect the specimen from dust during drying, the slide should be supported with the film side down. A wooden slide container may be used for the purpose. It is important to have the slide level, since otherwise the blood might collect at one side of the film and not be evenly distributed. From eight to twelve hours should be allowed for drying unless the process can be hastened by using an electric fan. In this procedure, the slide should be placed with the film side up in front of the fan. The distance from the fan should be such that the current of air will not pile the blood on one side of the film.

The slide should not be mailed until the films are entirely dry, and each slide should be wrapped in soft paper or tissue.

The sooner the specimens reach the laboratory after collection the better, since malaria parasites stain most characteristically in freshly prepared films.

If a sternal puncture is made, the films should be prepared in the same manner as blood films.

Measles

Measles is caused by a filtrable virus, but practical laboratory aids in diagnosis are not available at present. If the patient should develop symptoms of pneumonia or encephalitis, however, the examination of specimens described under Pneumonia, p. 60, or Encephalitides (Virus), p. 34, is desirable.

Products Supplied by the Laboratory

Immune serum globulin (human). At present, immune serum globulin (human) is being furnished to state health departments, without charge, by the American Red Cross for use only in the prevention or modification of measles. As yet no authority has been given to distribute it for the treatment of other diseases. Globulin solution is distributed in bottles containing 2.0 ml. and may be obtained by direct application to the Central Laboratory for immediate use in contact children under four years of age and, when indicated, in those in institutions. The limited supply makes it necessary to restrict its use to this age group. For either prevention or modification, the globulin should be administered within six days after the

initial exposure, but it will probably exert some effect until about the tenth day, particularly if the dose is increased. A modified attack of measles induces an active immunity.

Administration. A dose of from 0.5 to 1.0 ml. will usually modify the disease; 1.5 to 2.0 ml. will usually prevent it. The globulin should be injected intramuscularly, preferably in the buttock. Care should be taken to draw back on the plunger of the syringe before injection in order to be certain that the needle is not in a blood vessel. This preparation is not for intravenous use. Detailed directions accompany each package.

In order to secure data on the value of globulin solution, it is essential that reports be received on all cases in which it is used. A report form is enclosed in each package. It should be returned after three weeks to the Division of Laboratories and Research, New Scotland Avenue, Albany 1.

Sodium citrate solution for use in the collection of parent's blood. Published reports and those that have been received by the State indicate that whole blood taken from an adult with a history of measles is an effective prophylactic agent. It is used for preventive treatment of contact children between three months and five years of age, the period in which most of the deaths from measles occur. It may also be given in the case of older children whose physical condition is such that an unfavorable prognosis would be anticipated should measles develop.

Sterile sodium citrate solution for use in the collection of parent's blood for the modification or prevention of measles is distributed through the local supply stations. Each package contains sufficient solution for one preventive treatment, a circular giving detailed directions, and a report form.

The blood from one of the child's parents who has had measles should be used unless contraindicated. Blood grouping is unnecessary. Only persons in good physical condition and free from evidence of tuberculosis, syphilis, malaria, or any other communicable disease should be selected. The blood is taken from one of the veins at the elbow into a syringe into which the sodium citrate solution has previously been drawn. Aseptic precautions should be observed throughout the procedure.

Administration. The citrated blood is injected slowly into the muscles of the lateral aspect of the thigh, into the upper outer quadrant of the buttocks, or between the scapulae. It may be injected

in two areas. The dose for children under five years of age is from 20 to 30 ml. of blood. In case the blood is given to children over five years of age double the amount has been advised.

Physicians are urged to fill out and return after three weeks to the Central Laboratory in Albany the report form contained in each package of sodium citrate solution.

Meningitis and Meningococcemia

Meningococcus Meningitis. While many species of pathogenic bacteria have been reported as the occasional incitants of meningitis, in cases of purulent meningitis that do not follow an infectious process in the mastoid or elsewhere, the meningococcus *(Neisseria meningitidis)* is the microorganism most frequently encountered. Consequently, when the cerebrospinal fluid is cloudy, not as the result of a bloody tap, chemotherapy should be commenced promptly, in the absence of definite contraindications. The use of serum depends upon the bacteriologic findings and the clinical manifestations. Since meningococci autolyze readily few may be found in the cerebrospinal fluid, and in some instances, when the examination cannot be made promptly, none can be demonstrated. Experience has indicated that when large numbers of polymorphonuclear leucocytes are present in the cerebrospinal fluid and no bacteria are found, the incitant is usually the meningococcus, which is often found in a later specimen from the patient.

Influenza Meningitis. Recently, a considerable percentage of cases of meningitis, particularly in children, have been incited by *Hemophilus influenzae.* In New York State, in the majority of instances, the incitant has been classified as type b.

Meningococcemia (septicemia). Invasion of the blood stream by *N. meningitidis* may precede or occur independently of meningococcus meningitis. Chronic infection of the blood stream with this microorganism is not uncommon. Cultural examination of a blood specimen collected during a febrile episode, if the fever is intermittent, aids in establishing the diagnosis.

Meningococci may sometimes be demonstrated in freshly drawn blood by laking the cells and examining the centrifugate microscopically. This is especially true in fulminating infections in which prompt diagnosis is imperative. This procedure is not available for specimens submitted through the mail but only at a local laboratory.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, a specimen of cerebrospinal fluid in a sterile container (tube outfit—swab or needle removed) should be submitted for examination to a laboratory approved for the purpose. Directions for the collection of specimens are given under Syphilis, p. 83.

In meningococcemia (septicemia), the Sanitary Code also specifies that blood cultural tests should, if possible, be made in a nearby approved laboratory. Otherwise, 10 ml. of blood should be submitted (typhoid tube outfit).

Products Supplied by the Laboratory

Anti-Hemophilus influenzae type b serum. Meningitis due to *Hemophilus influenzae* is a disease primarily of infancy and early childhood, a large percentage of the cases occurring in patients under two years of age. Prompt diagnosis and early institution of therapeutic measures are of the utmost importance. Reports indicate, however, that in some instances satisfactory results have followed treatment of chronic forms of the disease. From available data, the administration of anti-Hemophilus influenzae type b serum (rabbit) together with one of the sulfonamide drugs, usually sulfadiazine, may effect recovery in a relatively large percentage of cases. According to reports, streptomycin either alone or in combination with immune rabbit serum and sulfadiazine is also an effective therapeutic agent.

The serum is produced by the Division of Laboratories and Research and dispensed in doses of approximately 25 mg. of antibody nitrogen, on which dosage is based. A limited supply is available for distribution to physicians for the treatment of *Hemophilus influenzae* type b meningitis in infants and children. The serum is supplied directly from the Central Laboratory. Requests should be sent by telephone or telegraph as soon as diagnosis has been established.

Administration. The serum therapy and chemotherapy of influenza meningitis are still not clearly established. An initial subcutaneous injection of sulfadiazine should be given at once if the spinal fluid shows a preponderance of polymorphonuclear leucocytes. Subsequent dosage and routes of administration depend upon the laboratory findings, the response of the patient, and drug-level requirements.

Serum may be administered as soon as the bacteriologic diagnosis has been made. Prompt and ample dosage is recommended. In mild cases, conservation of serum may warrant delay in intravenous administration for about twelve hours after the initial injection of sulfadiazine. In moderate and severe cases, the serum should always be given without delay. Intravenous administration is customary. The drip method has been advocated. In severe cases, supplementary intraspinous injections may be indicated. Favorable results have been reported in a limited number of cases following intramuscular administration. Directions for use and a report form are enclosed in each package. For precautions against anaphylactic reactions, see page 19.

Antimeningococcus serum. Serum therapy of meningococcus infections has recently been almost completely supplanted by sulfonamide and penicillin therapy. The results of chemotherapy are much better in these infections than those formerly obtained by serum therapy. However, antimeningococcus serum may be indicated in certain cases.

On special request, antimeningococcus serum in bottles containing 20 ml. prepared by the Division of Laboratories and Research is distributed by the Central Laboratory in Albany, the Branch Laboratory in New York City, and the laboratory supply station in Buffalo.

> Since a multivalent antimeningococcus serum is available, group differentiation is not essential. It is, however, of interest and may be of great importance to classify the particular strain, especially in refractory cases. In such instances, the local laboratory should be requested to send the strain isolated to the central laboratory for further study or, if local facilities are lacking, the spinal fluid should be sent directly.

Administration. The serum may be given intraspinously or intravenously under aseptic precautions. Both methods would appear to have a place in the serum therapy of meningococcus meningitis.

Full directions concerning precautions against anaphylactic reactions will be found on page 19. These directions and the technic of administration of the serum are also given in the circular that accompanies each vial.

For intraspinous injection in adults, from 20 to 40 ml. of the serum may be given; in children, up to 20 ml. or more. The amount that is introduced should be somewhat less than the quantity of cerebrospinal fluid withdrawn and should depend upon the ease

with which the serum runs in by gravity. Injections of the serum at 24-hour intervals are usually adequate when intraspinous administration is used. In severe cases, the serum may be injected every six or twelve hours for three or four doses and thereafter every twenty-four hours. In prolonged subacute or chronic cases, the administration must be continued. Intraspinous administration of serum for too long a period may give rise to symptoms that suggest recurrent meningitis. Overtreatment should, therefore, be avoided. The number of injections will depend upon the patient's general condition and the bacteriologic examination of the spinal fluid. Injections should, in general, be continued until at least two successive specimens are free from meningococci.

For intravenous injection, from 20 to 40 ml. of the serum may be given, occasionally more. Here also continued treatment depends upon the patient's general condition and the bacteriologic examination of the spinal fluid. The schedule of doses suggested for intraspinous injections may be used.

Some of the published reports recommended an excessive dosage which appears not only to be unnecessary, if sera of high potency and broad valency are used, but possibly even harmful, especially if continued for any length of time.

Mumps

Mumps is caused by a filtrable virus. Parotitis is the most frequent clinical manifestation of infection but orchitis, meningoencephalitis, pancreatitis, and other complications are relatively frequent. It is believed that meningoencephalitis is not uncommon in the absence of parotitis. An etiologic diagnosis in such cases requires laboratory examinations.

Specimens for Laboratory Examination

Mumps virus may be isolated from the saliva of patients with parotitis by the inoculation of embryonated hens' eggs. The saliva should be frozen in dry ice at the time it is obtained and shipped in the frozen state. The isolation of virus from spinal fluid of patients with meningoencephalitis is not practical. The cell count, protein determination, and colloidal gold test are of value. (See Methods for Collecting Specimens. Cerebrospinal fluid, p. 83).

The diagnosis of mumps meningoencephalitis is best accomplished by the simultaneous examination of acute- and convalescent-phase

sera in a complement-fixation test with mumps antigen. The convalescent-phase sera should be obtained approximately one and two months after the onset of illness. (See Methods for Collecting Specimens. Blood, p. 82).

Mycotic Infections

Actinomycosis. Actinomycosis, which is one of the most common of the mycotic diseases, may be incited by a number of species of actinomyces. The infection may be localized or generalized. Localization usually occurs in the head and neck (lumpy jaw), or in the abdominal and thoracic organs. The disease may spread by continuity or through the blood stream to any part of the body. It is characterized by formation of abscesses in the central area. The purulent material generally contains the "sulfur granule."

Pus from palpable glands, cutaneous or subcutaneous abscesses, or nodules should be aspirated under aseptic precautions with a syringe and needle. The specimen should be submitted in a sterile tube (tube outfit—without swab). Material from suppurating lesions should be collected on two or more sterile swabs (tube outfit —with swab).

When an actinomycotic lesion in the lung is suspected, sputum should be obtained with a bronchoscope, whenever possible. Otherwise, sputum from the deeper air passages, coughed up after a thorough rinsing of the mouth with a suitable antiseptic, should be submitted (jar outfit).

Cryptococcosis. Cryptococcosis may be subacute or chronic, involving almost any part of the body. The lesions are most frequently found in the brain and meninges. The etiologic agent is *Cryptococcus neoformans* (*hominis*) (*Torula histolytica*).

A specimen of cerebrospinal fluid should be submitted when the symptoms suggest involvement of the central nervous system. Directions for collection of the specimen are given under Syphilis, p. 83.

If the lesions are not located in the central nervous system, specimens of pus from subcutaneous abscesses or nodules, or sputum should be submitted, as described under Actinomycosis.

Moniliasis. The etiologic agent of moniliasis is *Candida (Monilia) albicans.* It may infect the skin, nails, mucous membranes (mouth and vagina), bronchi, and lungs, or even cause septicemia, meningi-

tis, or endocarditis. *Candida albicans* is often a secondary invader and is frequently found in the mouth and feces as a saprophyte. A diagnosis of pulmonary moniliasis should, therefore, be made only after repeated examinations of properly collected specimens of sputum have shown the presence of the microorganism, and tuberculosis has been definitely excluded.

In cutaneous moniliasis, scrapings from the infected area should be submitted in a sterile container (tube outfit—swab removed). Directions for collection of specimens are described under Ringworm.

Sputum should be submitted in a sterile container (jar outfit) as described under Actinomycosis.

Blastomycosis (Gilchrist's disease). Blastomycosis is a chronic disease that may be localized or systemic. The local or cutaneous disease begins as a small papulo-pustule, which becomes encrusted and eventually develops into a large granulomatous lesion with verrucous surface and a smooth border. The systemic form usually follows a primary pulmonary infection and may spread by the blood stream. Infection of the intestinal tract rarely occurs. The etiologic agent is *Blastomyces dermatitidis.*

Bits of tissue obtained by scraping with a scalpel should be collected from cutantous lesions and submitted in a sterile tube (tube outfit—without swab). Pus from subcutaneous abscesses should be aspirated under aseptic conditions with a syringe and needle and placed in a sterile tube, or material from suppurating lesions should be collected on two or more sterile swabs and submitted in sterile tubes (tube outfit—with swab). Sputum should be collected and submitted as described under Actinomycosis.

Histoplasmosis. Histoplasmosis is a generalized infection affecting essentially the reticuloendothelial cells. The etiologic agent, *Histoplasma capsulatum,* is found chiefly in the mononuclear cells of the peripheral blood, sternal bone marrow, lymph nodes, or spleen.

Films from the peripheral blood, sternal bone marrow, or material from a lymph node puncture should be submitted on glass slides (slide outfit).

Coccidioidomycosis. This disease may occur in two forms—an acute, self-limiting, respiratory infection (California disease, valley fever), or a chronic, usually fatal disease (coccidioidal granuloma) involving the cutaneous, subcutaneous, visceral, and bony tissues.

While endemic in some parts of the western United States, the disease is rarely encountered in the eastern sections. The etiologic agent is *Coccidioides immitis.*

Sputum, pus from subcutaneous abscesses, or exudates from cutaneous lesions should be collected and submitted, as described under Actinomycosis, for cultural examination and animal inoculation.

Tissue from biopsy or post-mortem examination should be collected aseptically and submitted promptly without preservative for cultural examination. Also, duplicate specimens should be sent in fixative for histologic study.

Sporotrichosis. The etiologic agent of the disease is *Sporotrichum* (*Rhinocladium*) *schenckii* (*beurmanni*). The microorganism can rarely be demonstrated in pathologic material. While the initial skin lesions may be so characteristic clinically as to present no difficulty in diagnosis, the infection in its more complicated forms may be confused with a variety of infections such as tularemia, coccidioidomycosis, or even tuberculosis. In such cases, a diagnosis can be made only on the basis of cultural studies.

Pus should be collected with a sterile syringe from an unopened subcutaneous nodule and submitted in a sterile tube (tube outfit—without swab), or if all of the lesions are open, material may be submitted on sterile swabs (tube outfit—with swab) for cultural examination and animal inoculation.

Dermatophytosis (ringworm infection). Ringworm is a superficial infection involving skin, nails, or hair, and is incited by a group of fungi known as the dermatophytes *(Microsporum, Trichophyton, Epidermophyton).*

To reduce contamination, lesions on scalp, skin, and nails should be washed thoroughly with 70-per-cent alcohol and wiped dry with sterile gauze, before a specimen is collected. Grease or excessive natural oil should be removed by rubbing the area with gauze soaked with benzene. If the lesion is widespread and has a well-marginated border, material scraped from the periphery is more desirable.

When the lesions are on the scalp (Tinea capitis), the scalp should first be examined under filtered ultraviolet light (Wood's lamp) for suitable areas from which to obtain the specimen. The *Microspora,* chief incitants of tinea of the scalp, show a brilliant green fluorescense, and the fluorescent hairs only should be epilated. Several hairs obtained by epilation with forceps, or a few stumps of hairs and

scales obtained by scraping the areas with a scalpel should be submitted in a sterile tube (tube outfit—without swab).

From lesions on the feet (athlete's foot) and hands, a specimen obtained by removing with scissors the roof of fresh vesicles, or by scraping with a scalpel around the edges of any fissure in the interdigital webs should be submitted (tube outfit—with swab).

Ophthalmia Neonatorum

Many cases of ophthalmia neonatorum, especially the severe ones, owe their origin to the gonococcus *(Neisseria gonorrhoeae)*. Proper treatment of such infections must be begun promptly if the sight is to be saved. Unless laboratory service is available in the locality so that specimens can be examined immediately, the laboratory findings are of value only for purposes of confirmation of the clinical diagnosis.

When conjunctivitis is found not to be incited by bacteria, a diagnosis of inclusion blennorrhea should be considered.

Specimens for Laboratory Examination

In accordance with the requirements of Chapter II, Regulation 9, of the Sanitary Code, films of the exudate from the eye on glass slides (gonorrhea slide outfit) should be submitted for examination to a laboratory approved for the purpose. The fresh exudate on a swab should be rolled out on the slide to avoid distorting the leucocytes. A microscopic examination only can be made when the specimen is submitted by mail. If local laboratory service is available, cultural tests often prove helpful.

The most satisfactory specimens for the diagnosis of inclusion blennorrhea are obtained by gently stroking the conjunctiva with a small, blunt curette. Light anesthesia with cocaine may be helpful. The material should be spread lightly on a clean slide (miscellaneous slide outfit) and allowed to dry in the air. The type of examination desired should be clearly indicated on the accompanying history form. Conjunctival epithelial cells rather than leucocytes are desired for the microscopic examination, and this fact should be borne in mind when the material is collected.

Product Supplied by the Laboratory

Silver nitrate solution. The immediate application into the eyes of new-born infants of a 1-per-cent silver nitrate solution, or

other agent equally effective in preventing ophthalmia neonatorum, is required by the Sanitary Code. The central laboratory distributes the silver nitrate solution to physicians through district supply stations in outfits containing two wax ampoules. Each ampoule has sufficient material for the treatment of one baby. Full directions for use accompany each outfit.

Plague

Plague is principally a disease of rodents, the incitant of which, *Pasteurella pestis,* is transmitted, except in the pneumonic form, by fleas and probably other blood-sucking insects. The microorganism is harbored chiefly by the rat, but ground squirrels and other rodents have also been shown to be the source of the infection. Plague has been proved to be endemic in wild rodents in some districts in California, and infected animals are occasionally reported in other western states. Thus, the possibility of the occurrence of the disease in New York State must be kept in mind. While suggestive findings may be obtained by morphologic examination of exudate from a bubo, the results of bacteriologic and animal tests are necessary to identify *Past. pestis.*

If a diagnosis of plague is considered, the district state health officer should be notified at once by telephone.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, the following material should be submitted for examination to a laboratory approved for the purpose: (1) a specimen of discharge or aspirated fluid, if a bubo is present (tube outfit with swab); (2) 10 ml. of blood (typhoid tube outfit); (3) in the pneumonic type of plague, a specimen of sputum (jar outfit). If possible, the specimens should be delivered to a laboratory by messenger.

Pneumonia

Although the sulfonamides and penicillin have removed the urgency for pneumococcus-type differentiation, this examination may still be desirable if the clinical course indicates the need for serum treatment.

Pneumococcus-type differentiation can be undertaken with sputum from the deeper air passages, and with blood, cerebrospinal fluid, pleural and other exudates, and stomach washings.

Local approved laboratory service should be used whenever avail-

able. If serum is to be administered, this fact should be indicated on the history form, and the specimen should either be delivered by messenger or sent special delivery.

Product Supplied by the Laboratory

It has been clearly demonstrated that, even though favorable results were obtained in the treatment of pneumococcus lobar pneumonia with type-specific antipneumococcus sera, the sulfonamides and penicillin offer more effective, safer, and more easily administered methods of treatment of pneumococcus infections. In a small percentage of these cases, either because of demonstrated resistance of the strain of pneumococcus causing the infection to the chemotherapeutic agent employed or because of evident failure of treatment for unknown reasons, the administration of serum may be indicated.

Antipneumococcus sera. The production of antipneumococcus sera, horse and rabbit, has been discontinued by the Division of Laboratories and Research. As long as the stocks of filled material are available, they will be furnished on special request from the Central and Branch Laboratories only. The horse sera and many of the rabbit sera are concentrated and purified. A small bottle containing normal horse or rabbit serum diluted 1:10 with salt solution for use in the tests of sensitivity to the serum is included in each package of antipneumococcus serum. Sterile physiologic salt solution in 10-ml. amounts may be obtained for use in preparing the dilution for the intracutaneous test of susceptibility, for rinsing water from syringes and needles that have been boiled, and for diluting the serum for the preliminary injection.

Every physician using antipneumococcus serum provided by the State is asked to report the results of its use on the form supplied with each package. The form should be filled out completely and returned to the Central Laboratory, Albany.

Administration. To be effective the serum must be given intravenously. Full directions concerning precautions against anaphylactic reactions will be found on page 19. These directions and the technic of administration of the serum are given in the circular that accompanies each bottle. Directions for the treatment of serum reactions other than anaphylactic are also given in the circular. Caution should be observed to avoid overheating of the serum in preparing it for injection.

A preliminary injection of 1 ml. of the therapeutic serum diluted with 10 ml. of physiologic salt solution may be followed, if not contraindicated, in from one-half to one hour by the remainder of the first dose, the contents of two vials. A second dose should usually be given from two to four hours later. For the treatment of the average case of type-1 pneumonia, the contents of four vials are recommended; for type-2, about eight vials. The contents of from four to six vials is suggested for the other types. At least double the dosage may be required when treatment has been delayed, in older patients, in those with positive blood cultures or complications, in patients who are pregnant, and in those who fail to improve within twelve hours after the first serum injection, provided the type diagnosis has been verified by the examination of a second specimen of sputum or a blood culture. Favorable clinical response is the most reliable index of adequate dosage. If chemotherapy is used in combination with serum therapy, smaller total amounts of both drug and serum may be required than are necessary when either is used alone. Sulfonamide drugs for the treatment of pneumococcus infections are no longer distributed by this Division since they can be purchased readily in the open market at a low cost.

Poliomyelitis

Poliomyelitis is caused by one of the smallest of the viruses. The agent is also unusual because of its stability. Nervous tissue may be stored for long periods in sterile 50-per-cent glycerol. Feces, an equally good source of virus, remain infective for long periods if refrigerated.

The most useful laboratory examination is that of the cerebrospinal fluid. To be of greatest value the specimen should be sent to a nearby laboratory. The findings obtained in the examination of cerebrospinal fluid do not distinguish poliomyelitis from other virus infections of the central nervous system. Many of these infections may be identified by specific laboratory tests, for which two or more blood specimens are required. Doubtful cases may be investigated by these means. Lymphocytic choriomeningitis, mumps meningoencephalitis, and the summer encephalitides may be so recognized. No significant serologic tests for poliomyelitis have been discovered. Increased cerebrospinal fluid protein suggests infectious neuronitis (Guillain-Barré syndrome), particularly if the cell count is relatively low.

Protozoan Infections
(Other than Amebiasis and Malaria)

Diseases incited by parasites have in the past been of relatively minor importance in New York State. In view of the large number of individuals who have recently been in countries where protozoan diseases are prevalent, these infections must now be kept actively in mind. Among infections incited by protozoa, amebiasis and malaria are of primary importance. Data regarding them are given under their respective headings. Other protozoan infections are mentioned briefly in this section.

Balantidiasis. Infection with *Balantidium coli* is common among hogs, and should, therefore, be considered when patients are known to have handled these animals.

Giardiasis. The incitant, *Giardia lamblia (intestinalis)*, has been reported as pathogenic, particularly for children.

Leishmaniasis. In the cutaneous form of leishmaniasis (oriental sore) and the Brazilian disease incited by Leishmania, the Leishmania may be found in exudate from the base of the lesion. In the visceral form, as in kala azar, the parasites may be found in the blood stream or in the reticuloendothelial cells of the various viscera. In the acute febrile stages, the parasites may frequently be found in thick blood films or in films prepared from the bone marrow.

Toxoplasmosis. Toxoplasmosis is particularly common in rodents, but has recently been recognized in man. Three types have been reported: congenital encephalitis and hydrocephalus; acute encephalitis in childhood; and a spotted-fever-like disease associated with atypical pneumonia in adults. The diagnosis is established by identification of the parasite in films from tissue or fluids. Specimens may also be submitted for animal inoculation.

Trichomoniasis. The most reliable laboratory aid in the diagnosis of vaginitis incited by *Trichomonas vaginalis* is the examination of moist discharge promptly after collection. If this cannot be done in a local laboratory, film preparations should be submitted. At least four films should be available so that examinations for both *Trichomonas vaginalis* and *Neisseria gonorrhoeae* may be made.

Trypanosomiasis. Two types, African sleeping sickness and Chagas's disease, have been recognized. In the acute febrile stages,

in both diseases, the trypanosomes may be found in the blood. They may be found also in tissue specimens. The lymph nodes may contain them in the case of African sleeping sickness, as well as the cerebrospinal fluid in the last stages of the disease. The parasites are found to be particularly numerous in the heart muscle in Chaga's disease.

Specimens for Laboratory Examination

Directions for the collection of feces specimens are given under Amebiasis, p. 22; those for the preparation of blood films, under Malaria, p. 49.

Examinations for evidence of protozoan infections can best be undertaken in a local laboratory.

Psittacosis, Ornithosis, and Pneumonitis

A number of viruses are known to cause pneumonia or pneumonitis in man. Four, psittacosis, ornithosis, S-F pneumonitis, and Louisiana pneumonitis viruses, are closely related. With the aid of laboratory tests, disease due to these agents can be differentiated from atypical pneumonias caused by other incitants. Differential diagnosis within the group and from infection with the closely related virus of lymphogranuloma venereum requires extensive laboratory study in many instances.

Infections with this group of viruses range from inapparent to fatal disease. Infection is frequently acquired by contact with birds, particularly psittacine birds (psittacosis) and pigeons, chickens, and ducks (ornithosis). In some areas many birds of one of these species are carriers. Infection may follow contact with patients. Physicians and nurses have become infected in this way. Penicillin is effective against psittacosis and ornithosis viruses.

The Sanitary Code, Chapter II, Regulation 37 prohibits the importation, breeding, sale, or offer of sale of birds of the psittacine family, with the exception that the importation and breeding of such birds for scientific research or exhibition in public zoological gardens may be permitted subject to the approval of the State Commissioner of Health.

Specimens for Laboratory Examination

The virus may be present in the sputum or blood of patients during early acute illness; occasionally it persists in the sputum after clinical recovery. Specimens should be shipped frozen in dry ice and the laboratory notified by telephone or telegram. Isolation and

identification of viruses of this group require the inoculation of animals or embryonated eggs.

When the examination of birds is desirable, they should be chloroformed, soaked in 5-per-cent lysol, wrapped in cloth or cotton soaked in the same antiseptic, and shipped to the laboratory by express in a water-tight container packed in dry ice.

Complement-fixation tests for psittacosis and ornithosis may establish a diagnosis if both acute- and convalescent-phase sera are examined and the results are interpreted in conjunction with the clinical course. Examination of single convalescent-phase specimens or serologic examination without clinical information is seldom helpful; a positive reaction may be due to prior infection or to infection with related viruses such as lymphogranuloma venereum, trachoma, or inclusion blennorrhea. Hence, two specimens of blood should be submitted, the first collected early in the illness and the second about two to four weeks later.

Rabies

Rabies is an acute and rapidly fatal infection of mammals, particularly dogs. The incitant, a virus, is present in the saliva of animals suffering from the disease, and may be conveyed through the broken or abraded skin, most frequently by bites of dogs. The period of communicability for man is not known, but for the dog it may be as early as four days before the onset of clinical symptoms and throughout the clinical course of the disease. The incubation period in man is usually from six to nine weeks, but it has been known to be as short as twelve days. In dogs, the period of incubation is usually fourteen days or less. Since, however, this period is sometimes prolonged, an animal that has been bitten should be killed or isolated under veterinary supervision for four months. An animal which is apparently normal but which has bitten a person should not be killed, but kept under competent observation for one week. If it shows clinical symptoms of rabies, it should be killed at once and the head submitted for laboratory examination (Sanitary Code, Chap. II, Reg. 10).

Since Negri bodies can usually be demonstrated microscopically in the dog's brain but very little earlier than the appearance of clinical manifestations, it is best not to kill the animal before such symptoms are evident. When Negri bodies are not demonstrated, animal inoculation, which usually requires from ten to thirty days for completion, is necessary.

Specimens for Laboratory Examination

Whenever any animal that has or is suspected of having rabies dies or is killed, it is the duty of the health officer to cause the head of the animal to be removed and sent immediately, properly packed, with complete pertinent data, to a laboratory approved for this purpose (Sanitary Code, Chap. II, Reg. 10). Great care should be taken to avoid infection from the dog's saliva, which may fleck its entire body.

It is important to avoid trauma to the brain tissue of animals to be examined for evidence of rabies. Strychnine or other chemical poisons that may interfere with the results of animal inoculation tests should not be used. The animals should preferably be killed by gas or by a shot through the heart.

A most important factor in the examination of the brain is the arrival of the specimen at the laboratory in a satisfactory condition. Decomposition renders the results of the examination, in most cases, unsatisfactory. The head should be submitted to the most accessible laboratory approved for the examination. (See p. 13.) Information in regard to the location of these laboratories is furnished to health officers and other physicians annually. The specimen should be kept cold, and, whenever possible, should be delivered by messenger. If messenger service is not available, the head should be placed in a water-tight container. This in turn should be put in a larger leak-proof container and packed with cracked ice. The use of dry ice is not recommended, since freezing of the brain, which usually occurs, delays the examination and may affect the condition of the tissue.

A limited number of outfits are available for shipment of animals' heads to the Central or Branch Laboratory to be examined for evidence of rabies. They are supplied through the district state health officers in whose territory rabies has occurred most widely. The outfit consists of a large outer container held tightly closed by two hasps fitted with key rings, and an inner 2-gallon, water-tight can for the head. There is sufficient space between the containers for ice. (See Fig. 5.)

Record of the clinical symptoms shown by the animal and information as to whether persons or other animals have been bitten or exposed should accompany the specimen.

An amendment to the Agriculture and Market Law, Article 6-A, Sections 105-c to 105-k, effective April 1, 1947, allows indemnification for domestic animals, defined as "domesticated sheep, horses, cattle, swine, and goats," dying of rabies. A report from an approved laboratory showing evidence of rabies is required before the owner can be paid for the loss of the animal.

FIGURE 4

OUTFIT FOR SHIPMENT OF HEAD OF ANIMAL TO BE EXAMINED FOR EVIDENCE OF RABIES

Products Supplied by the Laboratory

Rabies vaccine. Rabies vaccine is given for preventive purposes only. No effective therapeutic treatment is available. The prompt use of the vaccine is indicated in the case of all persons bitten by an animal with clinical or suspicious symptoms of rabies, of persons bitten by a stray animal that cannot be found, and in all instances in which the laboratory examination of the brain has shown the animal to have been rabid.

Cauterization. Wounds caused by rabid animals should be immediately and thoroughly cauterized by a physician with fuming or concentrated nitric acid. In districts where rabies is present all wounds caused by animal bites should be cauterized. Laboratory experiments in this country have indicated that cauterization by heat is less effective than by nitric acid and that carbolic acid, iodine, etc., are much inferior. Nitric acid should be applied very carefully to all parts of the wound and edges of the skin; for this purpose a glass rod is convenient.

Antirabic vaccine (Semple method), prepared commercially, is available to physicians in the State outside of New York City. Applications by telephone or telegraph should be made to the Branch Laboratory, 339 East 25th Street, New York City, 10. The name and age of the patient and the location of the bite should be given. It is expected that local boards of health in a position to pay for the vaccine will do so, or that they will arrange for reimbursement by the patient. Otherwise, the vaccine will be furnished by the State. Under a Federal ruling, commercial manufacturers of biologic products cannot sell their preparations directly to physicians. Therefore bills for rabies vaccine obtained through this Division must be made out to city, county, or local health departments. Except in special emergencies, requests to this Division for vaccine should be made by the health officer. Since rabies vaccine is not returnable for credit, it should be requested only for immediate use.

Sufficient vaccine for a course of fourteen daily injections is sent at one time. Immediately upon receipt, the material should be placed in the cold. Each dose is of equal strength and contained in 2 ml. Children receive the same dosage as adults. In the case of extensive bites and those on the head or neck, especially when the wound has not been thoroughly cauterized with strong nitric acid, or when treatment has been delayed, a course of twenty-one doses is suggested. The treatment should not be undertaken by

health officers or other physicians who are not familiar with it. The district state health officer should be consulted in any emergency, but if questions arise during the treatment it is advisable to communicate with the Branch Laboratory. Physicians who obtain the vaccine treatments from the State are expected upon completion of the treatment to fill out and return promptly to the Branch Laboratory the report form on the use of the vaccine, which is forwarded to them ten days after the material.

Administration. The injections are distributed in the subcutaneous tissue of the abdominal wall and the interscapular region. Since the virus is easily affected by temperature conditions and certain disinfectants, special care should be taken to follow the directions enclosed in each package. Some local soreness, together with erythema at the site of injection, may occur. Notice of other unusual symptoms, especially those of neuritis, should be sent promptly to the Branch Laboratory.

Note. The vaccination of dogs is now accepted as a valuable adjunct in the prevention and control of rabies. Under the provisions of section 25-a, Article III, of the Public Health Law, the State Commissioner of Health has the authority to prescribe conditions under which vaccinated dogs may be at large in designated areas certified for rabies. To promote vaccination programs, the State gives financial aid to counties for dog vaccination clinics.

Rat-Bite Fever

Rat-bite fever usually follows bites by rats, although other animals occasionally are involved. It is now generally believed that two types of the disease may occur, incited by *Spirillum minus* or *Streptobacillus moniliformis.* The clinical manifestations of these two infections frequently resemble each other so closely that they can be differentiated only by isolating the inciting microorganism. In either case, an indurated lesion usually develops at the site of the wound in from one to three weeks after the bite. It is followed by fever of a relapsing type and frequently by rash. Leucocytosis may occur, and a reaction may be obtained with the blood in serologic tests for syphilis. Both types of disease may respond to treatment with arsenicals, particularly that induced by *Spirillum minus.* Arthritis may be a predominant complication in the infection incited by *Streptobacillus moniliformis;* in this type penicillin is reported to be beneficial. In some instances, epidemics incited by the latter microorganism, unassociated with rat bite, have been recorded (Haverhill fever).

Specimens for Laboratory Examination

Spirillum minus remains viable for only a very short time outside the animal organism. Arrangements should be made to inoculate mice or guinea pigs with specimens of blood promptly after they have been collected. Serum expressed from the margin of the wound or from a skin macule, or fluid aspirated from the regional lymph node may also be examined.

Blood from the inoculated animals is examined for spirochetes by dark-field illumination daily from the eighth to the fifteenth day after inoculation. Cultural examination is also made for *Streptobacillus moniliformis* in case the animals die. Since special freshly prepared media are required, the director of the laboratory where the cultural examinations are to be undertaken should be advised in advance so that they will be available.

Rickettsial Diseases

Infections incited by microorganisms of the genus *Rickettsia* are characteristically transmitted through arthropod vectors. The following are the five rickettsial diseases which have been observed in the North Atlantic States, the causative agents, and the vectors:

Disease	Causative Agent	Arthropod Vector
Epidemic (European) typhus	*Rickettsia prowazeki*	Louse
Sporadic (murine) typhus (including Brill's disease)	*R. prowazeki;* also *R. typhi (R. mooseri)*	Flea
Rocky Mountain spotted fever	*R. rickettsia*	Tick
Q fever	*R. burneti*	Tick
Rickettsialpox	*R. akari*	Mite

A number of other rickettsiae are pathogenic for man, e.g., *R. orientalis,* the incitant of scrub typhus. As yet they are not known to have caused natural infection in New York State. Certain chemotherapeutic and antibiotic agents, including para-aminobenzoic acid, are effective in treating some of these infections.

The differential diagnosis between certain of the rickettsial diseases can sometimes be accomplished by a combination of clinical and epidemiologic data. Laboratory confirmation of the etiologic diagnosis is always desirable, however, and usually a differential diagnosis can be made only by such means.

Specimens for Laboratory Examination

The isolation of microorganisms of the rickettsial group requires the inoculation of animals or embryonated eggs. For this purpose, blood should be taken during the first days of the illness. It is desirable that such specimens be heparinized to prevent clotting and that they be refrigerated, preferably in dry ice.

Two types of serologic tests are available, agglutination with *Proteus* X strains and complement-fixation. The older, the agglutination test, depends upon the fact that certain bacteria of the *Proteus* group are agglutinated by the serum of patients with typhus or Rocky Mountain spotted fever. Repeated specimens are desirable to determine whether an increase in antibodies occurs during the course of the disease. These tests are of no value for the diagnosis of Q fever or rickettsialpox.

Complement-fixation tests are also available. Two specimens of blood should be submitted, the first collected as soon as possible after onset of illness and the other two to four weeks later. Results of complement-fixation examinations will establish the diagnosis of all of the rickettsioses and are more sensitive and specific than the agglutination test with *Proteus* X strains.

Note. A limited amount of Rocky Mountain spotted fever vaccine has for several years been supplied by the National Institute of Health to the Central Laboratory in Albany for use in New York State. In view of the small supply, it is necessary to restrict distribution to persons who are definitely exposed to bites of ticks in known infected areas. It is only recommended for prophylaxis, not for therapeutic use.

Smallpox

Smallpox is caused by variola virus. Vaccinia virus is a modified variola virus evolved by repeated passage through animals. The clinical diagnosis of smallpox may be difficult in mild, sporadic cases; characteristically the vesicles or pustules are nearly uniform in all areas of the body surface, while in chicken pox the lesions appear in successive crops. Laboratory examinations should be undertaken whenever smallpox is suspected.

Specimens for Laboratory Examination

The contents of two or more vesicles or pustules should be collected in sterile capillary tubes (chancre fluid outfit) after thorough cleansing of the skin with 70-per-cent alcohol. If vesicles or pustules are no longer present, several crusts may be submitted in a sterile container. Refrigeration is highly desirable during shipment, preferably

in dry ice, but is not essential. In this respect, variola virus differs from the majority of virus agents. Since animals or embryonated eggs must be inoculated, the results of laboratory tests are not available in less than four days.

Vaccination against Smallpox

State regulations relating to vaccination against smallpox—on approved methods of vaccination, reportability, care of cases, and the principal points of differential diagnosis between this disease and chicken pox—are given in the Sanitary Code, the Public Health Law, the Administrative Rules and Regulations of the State Commissioner of Health, and in pamphlets distributed by the State Department of Health.

State regulations prescribe that vaccine virus be kept at 40° F. or lower. The activity of the vaccine is materially affected by unfavorable storage conditions, and, undoubtedly, a majority of unsuccessful vaccinations might be traced to this source. The optimum temperature is about 10° below the freezing point. In no instance should vaccine that has been stored at room temperature be accepted.

A proper interpretation of the reaction following vaccination is essential. Vaccination properly performed with fresh, fully potent virus, will result in one of the three types of reactions: (1) typical primary vaccination, the usual course in an unvaccinated individual; (2) vaccinoid reaction, occurring in previously vaccinated persons, in which the broadest redness is reached in from three to seven days; (3) the so-called reaction of immunity, indicating full protection against smallpox, which reaches its maximum in from eight to seventy-two hours after vaccination. With vaccine that has lost any of its potency, however, varying reactions which may be confused with reactions of immunity occur, so that the proper interpretation can be made only by physicians thoroughly familiar with the several types of reactions, and when full potency of the virus used is definitely proved. A fully potent vaccine may be defined as one that gives one hundred per cent "takes" in previously unvaccinated individuals.

Product Supplied by the Laboratory

Smallpox vaccine, prepared commercially, is distributed by the Central Laboratory in 1-point packages through certain district laboratory supply stations for the use of physicians in their private

practice. Packages containing 10 points are provided for use in clinics at the request of district state health officers.

Snake Bite

The presence of rattlesnakes and copperheads in certain districts of New York State has, with the increase of camping and outdoor travel, become a matter of considerable popular interest and concern. While relatively few cases of snake bite are reported and fatalities in this part of the country have been rare, health officers and physicians should become familiar with the methods of treatment and the facilities now available.

Product Supplied by the Laboratory

Anti-snake-bite serum. A multivalent antitoxic serum is produced in horses against the venoms of the copperhead, water moccasin, and rattlesnake, three of the most poisonous snakes of North America. A limited supply of a commercial concentrated product is maintained at the Bear Mountain Headquarters of the Palisades Interstate Park, in the department of health at Copake, and at the district laboratory supply stations in Corning, Glens Falls, Kingston, Port Jervis, and Nyack. The serum is distributed for emergency use in the treatment of actual cases of snake bite, not for stock. If the patient is able to pay for the material or if it is a case covered by compensation, it is expected that the amount supplied will be replaced promptly. Physicians obtaining the serum are asked to send a complete report to the Central Laboratory in Albany.

In cases of snake bite the following procedure has been recommended: (a) the immediate application of a ligature or tourniquet a few inches above the bite, which is usually on the leg or arm, applied at first just tightly enough to prevent absorption and not interfere entirely with the flow of blood; (b) avoidance of exertion; (c) avoidance of all alcoholic or other stimulants. The tourniquet is released when serum has been given. Incision and suction are advisable to withdraw as much venom as possible, especially if treatment with serum is delayed. A dressing of a strong solution of table salt or Epsom salt in water may be used. Cauterization or the use of potassium permanganate is not advised. Detailed directions for the use of the serum are contained in each package.

Administration. The amount of restored serum from one ampoule (5 ml.) is stated to be usually sufficient to protect an adult against

the amount of venom injected by the bite of a moderate sized snake. In the case of children, however, double this amount is advised for the initial dose. If the patient's condition or the severity of the local manifestation indicates that a large amount of venom has been received, 30 to 60 ml. of serum should be injected at once. Injections are given preferably intramuscularly; a small amount of the serum may also be injected subcutaneously around the site of the bite. Intramuscular injection ensures more rapid absorption than subcutaneous. In late cases or in those in which puncture of a blood vessel at the time of the injury is suspected, the intravenous method is much to be preferred. It is highly important that the serum be given as early as possible. Under certain conditions, repeated injections at short intervals are recommended.

Streptococcus Infections

Hemolytic streptococci are associated with a variety of infections, including scarlet fever, erysipelas, septic sore throat, and puerperal sepsis. Although it is impossible by laboratory procedures to establish a definite etiologic relationship between a specific streptococcus and any of these conditions, the majority of cultures isolated from lesions in man belong to Group A (Lancefield). Thus, the group-precipitation test is especially useful in the study of hemolytic streptococci isolated from cows when outbreaks of streptococcus infection occur among consumers of raw milk from a particular dairy. Information regarding the serologic types of strains, that is whether the cases have been incited by one or more types, is also frequently of importance in the investigation of outbreaks. Experience is indicating that when hemolytic streptococci belonging to group A are isolated from milk, one of the milkers has a history of recent acute infection, such as sore throat, scarlet fever, or a lesion on his hand. Veterinary examination of the cows usually indicates that one or more has mastitis incited by streptococci from such a lesion. Occasionally the evidence points to the fact that the raw milk has been contaminated directly from the human source.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code with respect to streptococcus sore throat (including scarlet fever), a culture from the throat on Loeffler's blood-serum medium, and the swab used in making the culture (diphtheria

culture outfit) should be submitted for examination to a laboratory approved for the purpose. The diagnosis should be clearly indicated on the history form, as well as the fact that an examination for hemolytic streptococci is desired.

Investigation of outbreaks of scarlet fever or septic sore throat among users of unpasteurized milk from a common source should include a study of cultures collected from lesions on the hands and from the noses and throats of all persons coming in contact with the cattle or the milk, and the examination of samples of milk collected from the individual quarters from any animals in the herd that show evidence of mastitis or have lesions on the udders or teats. Samples of milk may be satisfactorily preserved for this type of examination by combining two parts of milk with one part of glycerol of tested purity.

Streptococcus cultures from outbreaks may be sent to the Central Laboratory for group and type determination.

Products Supplied by the Laboratory

Hemolytic Streptococcus Antitoxin (Antistreptococcus Serum)

Scarlet fever, erysipelas, etc. A hemolytic streptococcus specific to scarlet fever has not been differentiated. Scarlet fever, therefore, should not be considered a specific disease, but simply one manifestation of hemolytic streptococcus infection. Any one of the forty types of hemolytic streptococcus in Group A (Lancefield) that produces toxin can be considered an incitant of the disease.

Since the sulfonamides and penicillin became available almost the only indications for the use of antitoxin is to combat the toxemia of the severe cases of scarlet fever. The antibacterial action of the chemotherapeutic agents is believed to be more effective than that of the antitoxin. To prevent the suppurative complications of scarlet fever, chief reliance should be placed on penicillin. In erysipelas and other nonscarlatinal hemolytic streptococcus infections, penicillin has taken the place of the antitoxin as the most dependable therapeutic agent.

The concentrated and purified antitoxin supplied by the State Laboratory is produced by the immunization of horses with representative strains of hemolytic streptococci and their toxins. It is of high potency and neutralizes the toxins of almost all known types of Group A hemolytic streptococcus.

Administration. In severe cases of scarlet fever, early administration and adequate dosage are essential. The first dose should be at least 10,000 or 20,000 units; this should be repeated at intervals of twelve or twenty-four hours depending on the progress of the toxemia and the condition of the patient. For very young children smaller doses are adequate. Antitoxin and penicillin therapy may be given simultaneously. In scarlet fever, usually one dose, if sufficiently large, suffices. Satisfactory results are reported with intramuscular injection, but in severe cases it may be preferable to give part or all of the dose intravenously.

Streptococcus Toxin

Intracutaneous test of susceptibility. The intracutaneous test to determine susceptibility to a standard streptococcus toxin is performed similarly to the test for susceptibility to diphtheria toxin (Schick) and should be carried out with the same accuracy.

The test consists of the injection into the skin of one skin test dose of a standard streptococcus toxin. When a skin reaction develops, susceptibility to the toxin is indicated; a reaction measuring 10 mm. or over is considered positive. When the test is performed to determine whether immunity has been established after active immunization with the toxin, the dose of toxin used is also one skin test dose. A control injection of heated diluted toxin should always be made since some persons react to the protein in the material, especially after immunization.

The skin reaction appears much sooner with streptococcus than with diphtheria toxin and is usually much less marked. It develops within from six to twelve hours, usually reaches its maximum between twenty and twenty-four hours, and fades within forty-eight hours. A strongly positive reaction may occasionally be followed by pigmentation with very slight or no scaling. The readings should be made in a bright light from twenty to twenty-four hours after the injection. A circular giving directions for the use of the toxin and heated control and for the interpretation of reactions accompanies each package. The toxin is distributed through the district laboratory supply stations.

Active immunization. Streptococcus toxin for the active immunization of persons found by the intracutaneous test to be susceptible to the toxin is distributed only on special request. There is evidence that an immunity may be developed within two weeks following the

injections of the toxin, but how long this immunity will continue or how reliable it will prove is not known. Moreover, the large number of immunizing doses apparently required and the relatively large amount of toxic filtrate contained in them, would appear to make the treatment impracticable for general use. Under certain conditions, however, such as in outbreaks of scarlet fever in institutions or in the case of nurses in training, the use of the toxin may prove of value. If the individual immunized later develops hemolytic streptococcus infections such as scarlet fever or erysipelas, the fact should be reported.

For purposes of immunization, five and possibly six subcutaneous or intramuscular injections of increasing doses of toxin are given at 5- to 7-day intervals. Two weeks after the last injection, in order to determine whether active immunity has been established, the intracutaneous test of susceptibility should be repeated with one skin test dose of toxin. If the test still indicates susceptibility, the immunizing treatments may be continued. Directions for dosage and use accompany each package.

Syphilis

Laboratory tests are of the greatest importance in the diagnosis and evaluation of treatment of syphilis. Demonstration of the incitant, *Treponema pallidum,* is essential to early diagnosis. The director of a local laboratory is usually in the best position to collect fluid from the chancre for dark-field examination. When facilities are not readily available or the patient does not wish to be referred to a laboratory, the attending physician, if he is familiar with the procedure, can collect a specimen in an outfit containing sterile capillary tubes (Fig. 5) and submit it to an approved laboratory. Collection of fluid from the suspected chancre, in a laboratory, for immediate examination over a dark field is, however, the procedure of choice.

Examination for *Treponema pallidum.* If the lesion was treated with an antiseptic or for any other reason *Trep. pallidum* is not found, the aspiration of fluid from the enlarged regional glands should be undertaken. The study of such material is of particular importance when the primary lesion is in the mouth, since the morphology of certain of the mouth spirochetes resembles that of *Trep. pallidum* closely.

Treponema pallidum can usually be found in the initial lesion or in the regional glands as soon as the chancre develops, while a sero-

logic reaction often is not obtained until several weeks after infection. Thus, the importance of careful search for the inciting microorganism cannot be overstressed.

In case *Trep. pallidum* is not demonstrated in a suspicious lesion, repeated dark-field examinations should be made on several consecutive days, and blood for serologic tests should be submitted.

Examination of blood. During the secondary stage of a syphilitic infection, the blood reacts in almost one hundred per cent of cases. Thus, at a time when the disease is especially communicable, serologic tests are most dependable as an aid in diagnosis.

In the tertiary and latent stages, the percentage of reactions obtained is lower than in secondary syphilis. However, many such cases that would otherwise be overlooked are detected by means of serologic tests. Experience has shown that patients who require medical examination should have a serologic test for syphilis made. Legislation has been enacted that requires the submission of specimens from pregnant women (Public Health Law, Art. II-A, Sec. 18-d) and from applicants for a marriage license (Domestic Relations Law, Art. III, Sec. 13-a).

Specimens for serologic tests for evidence of syphilis should not be collected when the patient is acutely ill with some other disease or has recently been vaccinated.

Serologic tests for evidence of syphilis performed in well-conducted laboratories are, in general, so satisfactory that physicians have come to rely upon laboratory findings as an almost infallible aid in diagnosis. Not infrequently the results of laboratory examinations may be the only clue to the presence of a syphilitic infection. Consequently, an incorrect or misleading report of a serologic test for syphilis may be especially damaging. Such a report may be due to a mistake, for example, an incorrect name or an interchange of specimens, or to a clerical or technical error. Hence, a second specimen should be sent at once whenever the results of serologic tests are not in accord with other data concerning the patient. If similar findings are again obtained and no error can be discovered, other reasons should be sought.

Specimens from patients with yaws, pinta, and other diseases incited by treponemata or spirochetes react in serologic tests for syphilis. Such findings should not be classed as false positive reactions. Positive reactions have been reported as occurring more or less frequently with specimens from patients with malaria, leprosy,

pneumonia, tuberculosis, and other febrile diseases, and also from persons being immunized against smallpox and rickettsial infections. These reactions are largely dependent on the antigen and technic employed. Tests with antigens composed of the purified substances, cardiolipin, lecithin, and cholesterol, are less subject to these reactions than those using tissue extract antigens. Rarely, positive reactions may occur with specimens from apparently healthy persons. The blood of some individuals seems to be especially subject to changes that induce positive reactions. These usually occur in serologic tests that have not been adjusted to avoid oversensitive reactions. Recent literature indicates that reactions in serologic tests for syphilis may be obtained with specimens from some individuals who have been blood donors. This possibility should also be considered when reactions occur with specimens from patients who have had a severe hemorrhage.

If there is doubt that the patient has syphilis, such a diagnosis should be postponed for, at least, two or three months, especially when normal findings have been obtained on examination of the cerebrospinal fluid and a chest x-ray shows no evidence of syphilitic involvement of the large blood vessels. The outstanding exception to this rule is the pregnant woman whose blood reacts markedly. Treatment must be begun without delay to avoid the possibility of the birth of a syphilitic infant.

When adequate antisyphilitic treatment has been given during pregnancy, the blood from the umbilical cord may react in complement-fixation tests even though the baby does not have syphilis. Such reactions are considered due to the reagin derived from the mother. When the baby has no symptoms of congenital syphilis and the mother has had adequate treatment during pregnancy, antisyphilitic treatment is not advised until the infant's blood has been examined at intervals of a few weeks. If congenital syphilis has not been acquired, the reaction will be less with each succeeding specimen and after, at most, two or three months, the findings will be negative. In some instances reactions are not obtained in precipitation tests with specimens from the infant whose blood at first reacts in the complement-fixation test.

Quantitative complement-fixation test. The degree of reactivity in serologic tests is as important in syphilis as it is in other infectious diseases, and especially since clinical signs are so often lacking or indeterminate. The quantitative complement-fixation test used in

the Central and Branch Laboratories and in some of the approved laboratories titrates the reactivity of the syphilitic serum. The titer as reported represents the degree of the reaction. Equivalent values for any two serologic technics can be only approximate. In general, titers up to 3 correspond to slight (± or +) reactions; 4 to 6, to partial (2+ or 3+) reactions; and 7 or greater, to more marked (4+) reactions. Titers reported as greater than 10 represent a very wide range of strong reactions, that is, from slightly over 10, such as 11 or 12, to extremely high titers of 300 to 500, and even up to 2000. Slight variations in the titers, such as may be found in the examination of duplicate or confirmatory specimens, are of no significance. In the majority of instances, however, a titer that is more than 20-25 per cent greater or less than that found in the examination of a previous specimen represents an actual change in the reaction of the patient's blood.

Low titers can be expected in recently acquired syphilis, with specimens from patients under treatment, and from some of those with infections of long standing.

Marked reactions are almost invariably obtained with specimens from patients during the secondary stage of syphilis and from a considerable percentage of those with untreated syphilis of long standing. In case of a marked reaction, even when history or clinical evidence of syphilis is not obtained, treatment should be considered if the reaction is marked in subsequent specimens. A specimen of cerebrospinal fluid should be examined for evidence of neurospyhilis.

Quantitative precipitation test. A quantitative precipitation test employing the cardiolipin-lecithin-cholesterol antigen is useful for following the response to antisyphilitic therapy and for detecting relapse or reinfection. While the titers bear no mathematical relationship to the titers obtained in the complement-fixation test, they do afford a quantitative estimation of the reactivity of the patient's serum. The serologic patterns obtained by the two tests are generally similar.

Examination of cerebrospinal fluid. While the value of examing the blood is now universally appreciated, the necessity for the study of cerebrospinal fluid may not be so generally recognized. The detection of beginning neurosyphilis, or the ability to assure a patient, after he has had adequate treatment, that his cerebrospinal fluid is entirely normal is of vital importance. Thus, the cerebro-

spinal fluid of every patient who has been found to have syphilis should be examined at least once after treatment and a period of observation. Of course, at any time, in case neurologic symptoms of syphilis develop or the blood of the patient continues to react after he has had intensive treatment (this not infrequently occurs when there is involvement of the central nervous system), the study of the cerebrospinal fluid is imperative.

The director of a local laboratory is in a strategic position to assist the clinician through the study of specimens of cerebrospinal fluid. Such a study should include:

1. Macroscopic appearance
2. Determination of the cell content
3. Estimation of the protein content
4. Serologic tests (the results obtained with the cerebrospinal fluid should be compared with those secured with a specimen of blood collected on the same day)
5. Colloidal gold reaction

The results of laboratory tests for evidence of syphilis should be interpreted in the light of the clinical signs and history. Whenever they are at variance with other data concerning the case, specimens for confirmatory examination should be taken, to exclude the possibility of a mistake having been made. Treatment should never be undertaken when the only indication of syphilis is a slight degree of reaction in the complement-fixation test.

Specimens for Laboratory Examination

Chap. II, Reg. 9, Sanitary Code, requires submission to a laboratory approved for the purpose: (1) fluid from the lesion to be examined for *Treponema pallidum* (chancre fluid outfit containing capillary tubes, Fig. 5); (2) 10 ml. of blood for the complement-fixation test (syphilis tube outfit); (3) when laboratory tests fail to disclose evidence of syphilitic infection, 10 ml. of blood for the complement-fixation test, taken at weekly intervals until eight weeks have elapsed following the appearance of the primary lesion, unless evidence of syphilis is obtained earlier; (4) when syphilis of the central nervous system is suspected or before any syphilitic patient is discharged as arrested or cured, 5 ml. of cerebrospinal fluid for the complement-fixation test and other tests for abnormalities; with each specimen of cerebrospinal fluid, 10 ml. of the patient's blood, taken at the time

the specimen of cerebrospinal fluid was obtained for examination (Fig. 7).

For examinations required when blood or blood derivatives are to be used for therapeutic or prophylactic purposes, see Sanitary Code, Chap. IV-A.

Methods for Collecting Specimens

Chancre fluid. The lesion should be washed with sterile physiologic salt solution, and rubbed firmly with sterile gauze (a compress of 2 percent novocaine applied for a few monutes will aid in obtaining the deep exudate). After the blood has been removed, the tissues at the base of the lesion should be gently compressed until a drop of clear serum exudes on the abraded surface. The specimen can then be collected by touching this drop with the end of the capillary tube, which should be held in a horizontal position with the opposite end open. The serum or plasma will then rapidly enter the tube by capillary action. It should be sealed by pressing each end into the wax in the amber glass vial accompanying the outfit. While this is done, the tube should still be held in a horizontal position. (See Fig. 6). Repeated tests are desirable, since failure to demonstrate the spirochetes does not exclude syphilis. If an antiseptic or other local treatment has been administered, a salt-solution compress can be applied and the patient instructed to return on successive days for the collection of specimens or, in case the regional glands are enlarged, a specimen may be aspirated from them. A specimen should always be examined from the latter site when the chancre is located in the mouth, or when there is a question of mixed infection or balanitis. The physician collecting such specimens should, of course, protect himself by wearing rubber gloves.

Fluid from lymph nodes. When material is to be collected from the regional lymph nodes, those which are indurated, shotty, and not tender should be chosen. A 1-2 ml. syringe and a 22- or 24-gauge needle should be used. The needle is inserted well into the gland and its point rotated to break apart some of the tissue. A little of the fluid should then be withdrawn for examination. While collecting the specimen, the gland should be immobilized by grasping it so that the skin is drawn tightly over it. Care should be taken to have the point of the needle enter the gland and not the surrounding tissues. The aspirated fluid (which should contain very little blood) may then be deposited from the syringe upon a clean glass surface such as that of a slide or the side of a flat bottle, and collected in capillary tubes in the manner described for fluid from a chancre.

Blood. Specimens of blood, approximately 10 ml., should be taken preferably in the morning before breakfast or at least not within three or four hours after a meal.

If a blood-letting needle is used, the stylet should be removed and the needle attached to the sterile tube, precautions being taken to avoid contamination of the needle, the cork, or the inner surface of the tube. (See Fig. 8). The needle should be returned to the laboratory in the envelope provided for this purpose.

Syringes for the collection of blood should be sterilized by heating and permitted to cool before use. If a syringe has been boiled, it should be rinsed in sterile physiologic salt solution (about one-half a teaspoonful of salt to a glass of water). The blood should be transferred to the sterile tube immediately, before it coagulates in the syringe or needle. The tube should be left undisturbed in a slanting position at room temperature for one-half hour.

Cerebrospinal fluid. Proper collection of the specimen is of particular importance. The collection of no more than 5 ml. of fluid can be recommended as a routine procedure.

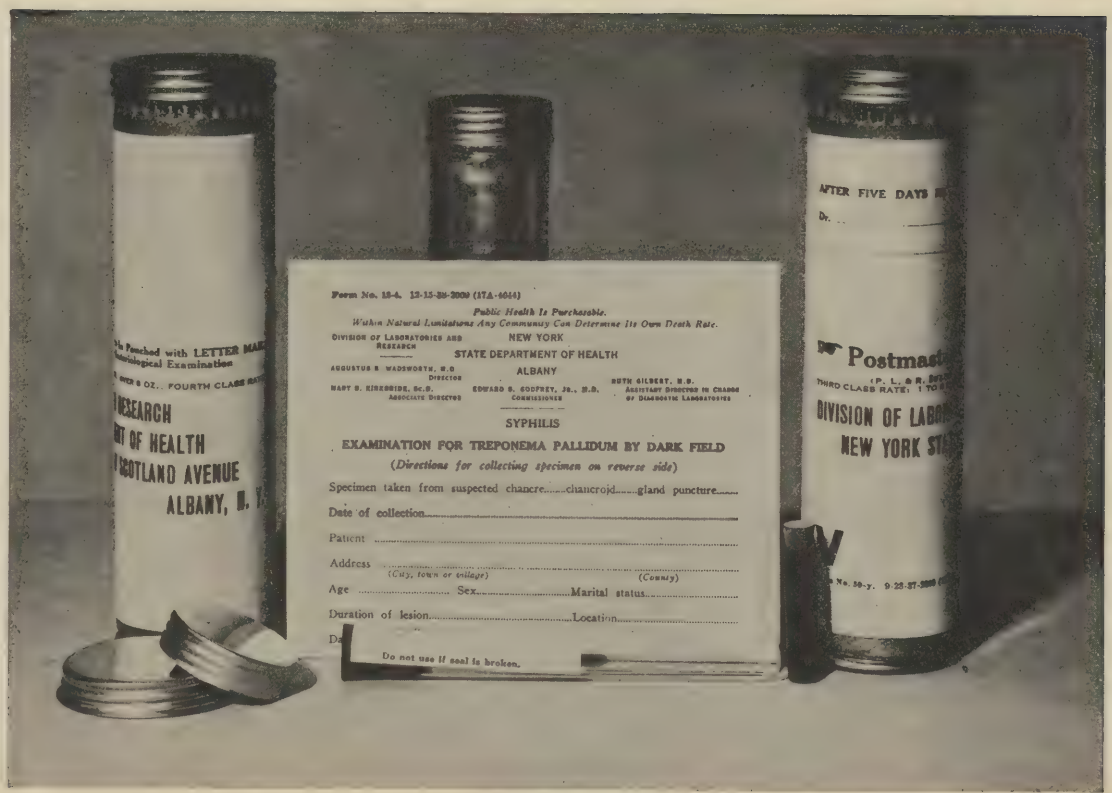
Since determination of the pressure of the cerebrospinal fluid is not necessary in an examination for evidence of syphilis of the central nervous system, apparatus for this purpose need not be used, thus lessening the chance of contamination. When a lumbar puncture is made, two needles, thoroughly cleansed and sterilized, preferably, in dry heat, should be available. They should have been carefully sharpened, since the use of a dull needle is usually responsible for admixture of blood in specimens of cerebrospinal fluid. If there is evidence of blood in the fluid, the tap should be discontinued and another puncture made with a fresh needle in the next interspace above the one that has been entered. Blood, oil, or any other foreign material in the cerebrospinal fluid usually renders it unsatisfactory.

Centrifugation of cerebrospinal fluid before submission for examination is most undesirable. In the case of a bloody tap, should most of the cells be thus removed, sufficient blood serum may remain, undetected, to affect the result of the serologic test. In the event that the specimen is from a syphilitic who does not have syphilis of the central nervous system, a reaction might occur when negative findings would have been obtained had the cerebrospinal fluid been uncontaminated with blood; that is, a reaction is obtained with the reagin in the blood that has contaminated the cerebrospinal fluid. Also, study of the cellular elements in cerebrospinal fluid may yield information of great value in diagnosis. Consequently, it is important that the whole specimen as collected be available for laboratory tests.

Products Supplied by the Laboratory

Arsenical and bismuth preparations and penicillin. Arsenical and bismuth preparations are purchased and distributed to physicians and clinics through most of the district laboratory supply stations. The drugs are at present supplied in the following amounts: oxophenarsine hydrochloride (mapharsen); bismuth salicylate in oil, 30-ml. bottles. Procaine penicillin, 3,000,000-unit bottles, is purchased and distributed through certain health departments and district laboratory supply stations upon request of the district state health officer for the rapid treatment of suitable cases of syphilis. Distilled water is also distributed for use by physicians and small clinics when not otherwise available.

FIGURE 5

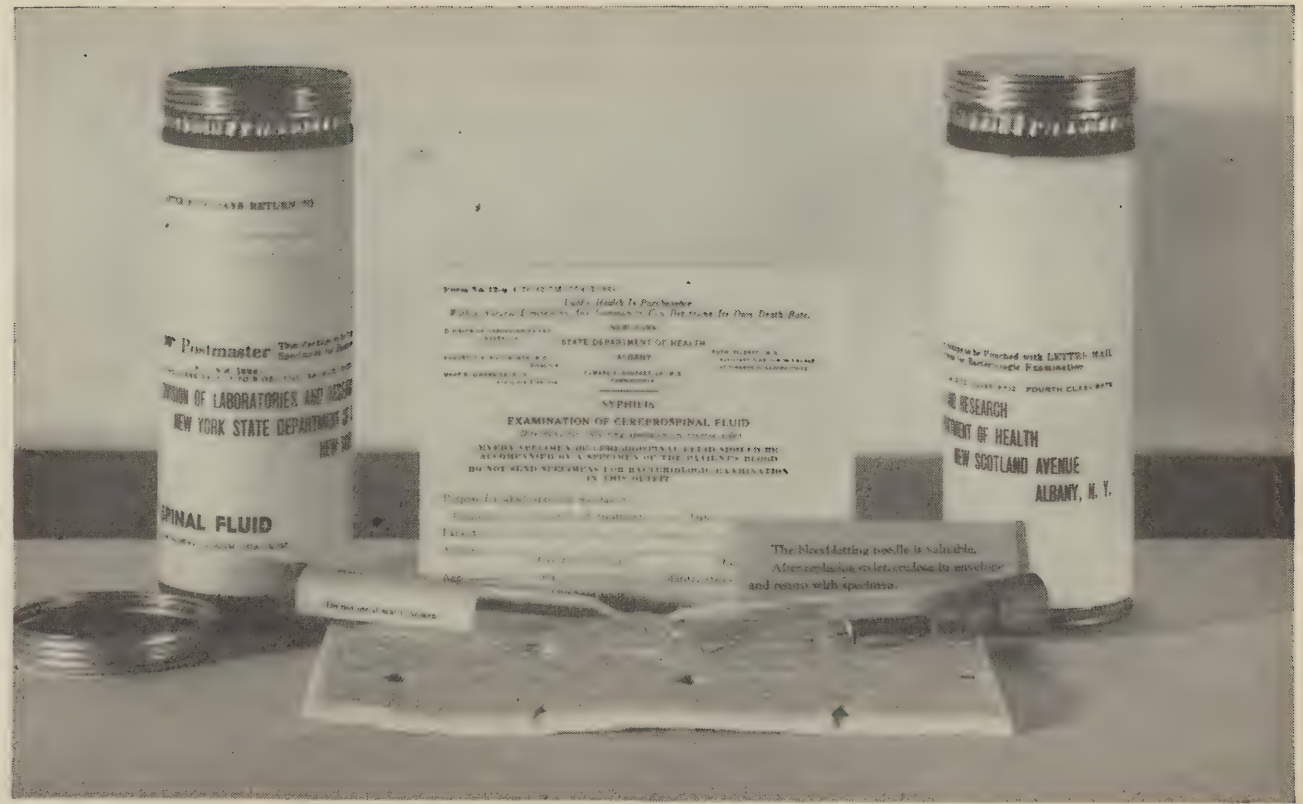


CHANCRE FLUID OUTFIT CONTAINING CAPILLARY TUBES

FIGURE 6

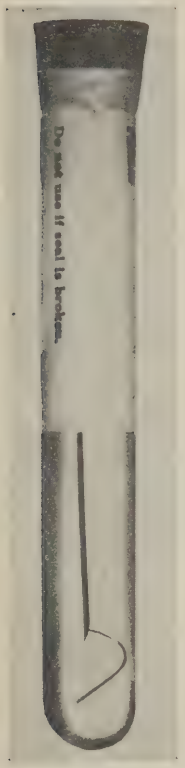
SEALING CAPILLARY TUBE WITH WAX

FIGURE 7

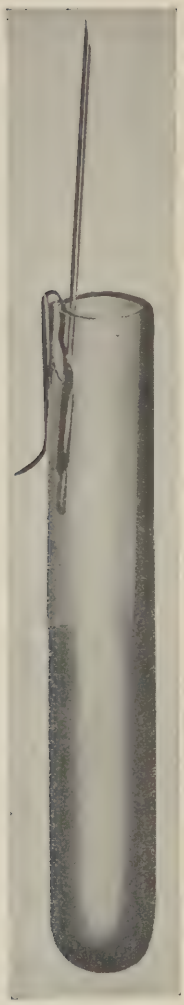


OUTFIT FOR BLOOD AND CEREBROSPINAL FLUID TO BE EXAMINED FOR EVIDENCE OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

FIGURE 8



A



B

TUBE FROM SYPHILIS OUTFIT

A. WHEN CORK IS REMOVED, LABEL IS BROKEN

B. ATTACHMENT OF BLOOD-LETTING NEEDLE WHEN SPECIMEN IS COLLECTED

When all necessary precautions are observed, serious reactions associated with the administration of these arsenicals are rare. Any unusual reaction occurring during or after injection should be reported immediately and in detail to the Central Laboratory. The kind of material given and the lot number should be specified. Instructions for the preparation of the solutions and the method of administration will be found in the circulars enclosed in each package.

Dimercaprol (BAL) is distributed through certain district laboratory supply stations for the treatment of cases of arsenical poisoning. Directions accompany each package.

Tetanus

Tetanus, like diphtheria, is essentially an intoxication. The tetanus bacillus (*Clostridium tetani*) grows only at the site of inoculation, usually a wound into which some infectious material such as soil contaminated with animal excretions has been forced. In dirty wounds, the microorganism finds favorable conditions for its development and produces one of the most powerful toxins known. A certain latent (incubation) period, proportionate in length to the distance of the portal of entry from the central nervous system, elapses before symptoms appear.

Specimens for Laboratory Examination

As considerable time may be required for the demonstration of *Cl. tetani* in the exudate of an infected wound or in a foreign body removed from the wound, the laboratory can be of little assistance in making an early diagnosis.

Products Supplied by the Laboratory

Tetanus Toxoid

Tetanus toxoid has been used extensively with excellent results for the protection of members of the armed forces. Its general use among adult civilians is not indicated. However, active immunization of children and of farm laborers and workers in certain industries, who are subject to repeated injuries, may be of definite value.

Tetanus toxoid, unprecipitated, contains no horse or other serum. It is distributed in 10-ml. and 2-ml. vials through district laboratory supply stations. When toxoid is requisitioned, the number of per-

sons to be injected should always be given. (See Diphtheria-tetanus toxoid, precipitated, p. 31.)

Administration. It is recommended that 3 injections of 1 ml. of tetanus toxoid, unprecipitated, be given at 21-day intervals. The intervals between injections may be somewhat lengthened if more convenient, but a shorter interval is not advised. Injections are given subcutaneously, alternately on the outer side of the upper arm, beginning with the left arm. Directions for its use are contained in each package.

In order to maintain an adequate level of immunity, a stimulating dose of 1 ml. of tetanus toxoid should be administered at the end of a year. A stimulating dose of toxoid at the time of an injury for which ordinarily a prophylactic injection of tetanus antitoxin would be given, is considered sufficient to protect against tetanus infection. In case of any doubt as to previous active immunization with tetanus toxoid, a prophylactic dose of tetanus antitoxin should be administered.

Tetanus Antitoxin

Passive immunization. Experience has shown that the subcutaneous injection of an immunizing dose of tetanus antitoxin rarely fails to prevent the development of the disease. When injury, resulting in a lesion favorable for the growth of tetanus bacilli, has occurred in a person whose immunization against tetanus is questioned, debridement by an experienced surgeon and a preventive subcutaneous injection of the antitoxin should be given as soon as possible. This is especially important in the case of gun shot or similar wounds or wounds in which garden, street, or stable dust or dirt has come in contact with the injured tissues. While one injection is generally sufficient, if the condition of the wound continues favorable for the development of tetanus infection, an additional subcutaneous injection should be given within five days and, under exceptional circumstances, even a third. The concentrated and purified antitoxin prepared by the State laboratory is available through the district laboratory supply stations in packages containing 1,500 units. A circular of directions is contained in each package.

Curative treatment. While the typical symptom complex of tetanus is unmistakable, the early evidences of the disease are frequently overlooked. Since to be of value it is essential that antitoxin be administered at the earliest possible moment, even brief delay in diagnosis or in treatment may remove all possibility of recovery.

By the time the first symptoms appear, the disease is well advanced and all that can be reasonably expected of the treatment is the prevention of absorption of further amounts of active toxin by the nervous system. At the onset any tetanus antitoxin available in the local supply stations, whether intended for treatment or immunization, should be used and an additional supply requisitioned at once by telephone or telegraph from the Central Laboratory or from the Branch Laboratory in New York City. The antitoxin is distributed in packages containing 20,000 units for therapeutic use. A circular of directions is contained in each package.

Administration

Prophylactic dose. The initial preventive dose is 1,500 units of antitoxin injected subcutaneously. For young children from 800 to 1,000 units may be given. In the case of a deep-seated and necrotic wound that has failed to heal, or a more superficial gun shot or similar wound, the injection should be repeated within five days after the initial dose. When the condition persists, a third and even a fourth injection after a similar interval may be advisable. In especially bad wounds larger and repeated doses may be needed. Should more than three days elapse between injections, the danger of severe anaphylactic shock should be borne in mind.

Therapeutic dose. The antitoxin may be administered intravenously, intraspinously, and intramuscularly. The intravenous and intraspinous methods are generally recognized as far superior to the intramuscular for the initial injections. There is considerable difference of opinion as to which is the more effective route. On the basis of reports received on cases treated in the State, combined intravenous and intraspinous administration appears to possess an advantage. Antitoxin should be administered at the earliest possible moment and in adequate amounts. Treatment should be continued depending upon the clinical signs, using intramuscular administration unless the severity of the symptoms requires continuance of the intravenous and intraspinous treatment. A large intramuscular dose distributed among several muscles should be given at once if the first intravenous and intraspinous injections are unavoidably delayed. Administration by cisternal puncture has been recommended.

(a) Intravenous injections of from 20,000 to 40,000 units repeated at 24- to 48-hour intervals.

(b) Intraspinous injections of from 10,000 to 40,000 units repeated at 24- to 48-hour intervals.

(c) Intramuscular injections of from 10,000 to 20,000 units.

For precautions against anaphylactic reactions, see p. 19.

Tuberculosis

The demonstration of tubercle bacilli in sputum or gastric washings is the most important laboratory procedure in the diagnosis of tuberculosis. As the search for cases has been intensified, laboratory methods have of necessity been sharpened to detect the causative microorganisms in specimens in which they are too infrequent to be observed by direct examination. Cultural methods have, therefore, come to be used widely, and experience has shown that they are of genuine value. The number of specimens in which tubercle bacilli are found when microscopic examinations are supplemented by cultural procedures is usually more than doubled. Guinea pigs are inoculated when indicated.

Pulmonary tuberculosis may be suspected from the x-ray and clinical findings, but can be diagnosed with certainty only by demonstrating the presence of tubercle bacilli.

Specimens for Laboratory Examination

Sputum coughed from the deeper portion of the respiratory tract, preferably in the morning, or exudate from lesions believed to be tuberculous, may be submitted in jar outfits without preservative, to be examined for tubercle bacilli. (See Fig. 9). For cultural examination, at least 25 ml. of sputum collected over a period of not less than forty-eight hours should be obtained. The examination of specimens collected at intervals of a few days is desirable when acid-fast bacilli are not found. The study of stomach washings is also of great value, especially in the case of individuals who do not expectorate. It is also important in following the progress and treatment of a patient, particularly just prior to discharge.

In tuberculosis of the intestines, examination of fecal specimens usually provides information of less diagnostic significance than clinical and x-ray findings. Patients with pulmonary tuberculosis often swallow sputum, and thus the finding of tubercle bacilli in

feces may not be indicative of a tuberculous involvement of the intestines.

When the patient has symptoms of tuberculosis of the kidneys, specimens of urine collected aseptically from each ureter should be examined.

The results of laboratory examinations may be helpful in confirming the diagnosis when a patient has symptoms of tuberculous meningitis. In most instances, a fibrin web collects in cerebrospinal fluid from patients with this disease. The web entraps nearly all of the tubercle bacilli present. If the specimen is sent through the mail, the possibility of finding tubercle bacilli by microscopic examination alone is materially lessened, since the web is usually broken and its remnants may adhere to the cork in the tube. The results of cultural examinations or of animal inoculations with specimens of cerebrospinal fluid from patients with symptoms of tuberculous meningitis are of value only for purposes of confirmation of the diagnosis. They would seldom, if ever, be available in a shorter period than three or four weeks. The study of specimens of feces, urine, and cerebrospinal fluid should be undertaken in local laboratories where all of the factors concerned can be evaluated.

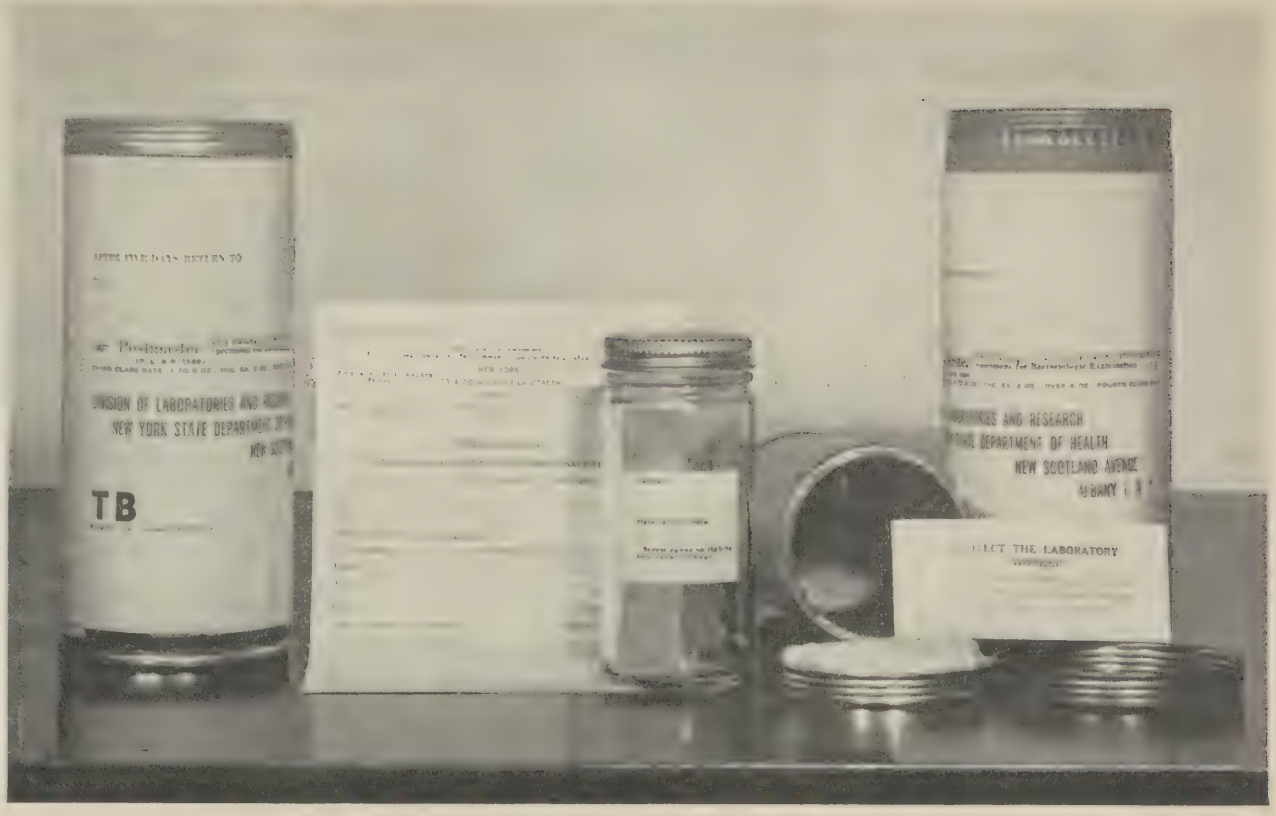
Products Supplied by the Laboratory

Old tuberculin (Koch's O. T.).

The tuberculin test is designed to determine the presence of tuberculosis infection. Physicians are cautioned, however, that although the results of the tuberculin test reveal the fact that the tissues have been sensitized by the tubercle bacillus, they should not be considered diagnostic evidence of clinical disease unless carefully interpreted in the light of considerable practical experience with the test and correlated with clinical findings.

Concentrated old tuberculin for diagnostic use is prepared by the Division of Laboratories and Research and distributed through supply stations to physicians experienced in making the test. The amount supplied is sufficient for a large number of tests. Vials containing material for individual tests are not distributed. Sterile salt solution for making the dilutions from the concentrated tuberculin is not furnished since it should be freshly prepared. The number of individuals whom it is planned to test should always be stated. The test may be made by the intracutaneous method (Mantoux), or by the cutaneous method (von Pirquet). Because of its greater

FIGURE 9



JAR OUTFIT (4 OZ.). TUBERCULOSIS

accuracy, the intracutantous method is recommended. The directions enclosed in each outfit should be followed closely.

Intracutaneous test. The tuberculin is diluted with sterile, freshly prepared salt solution so that the required dose is contained in 0.1 ml. Great accuracy should be observed in making the dilutions. For the initial test of children under eight years old, 0.1 ml. of a 1:10,000 dilution is advised; for older children and adults, 0.1 ml. of a 1:1,000 dilution. The dose is injected intracutaneously on the anterior surface of the left forearm. The reaction is considered positive when an infiltration and hyperemia develop at the site of injection in from six to eight hours. They reach a maximum in from twenty-four to forty-eight hours and then gradually subside. Readings should be made at 24- and 48-hour intervals. If no definite reaction is obtained after the first injection, especially in a case that according to the history or clinical symptoms is suggestive of active tuberculosis, the injection should be repeated with a lower dilution.

Cutaneous test. Two small scarifications one-fourth inch long and three inches apart are made, without drawing blood, on the left forearm. On one scarification a drop of the concentrated tuberculin is placed with a sterile needle, and is then spread gently over the surface. The second scarification is not treated and serves as a control. An inflammatory reaction developing where the tuberculin was applied and distinct from any traumatic reaction in the control area, constitutes a positive reaction. This usually appears in from twelve to twenty-four hours and subsides after from three to four days.

BCG vaccine

Vaccination with BCG vaccine is an effective and harmless method of inducing resistance to tuberculosis. The immunity is relative and vaccination is not a substitute for the segregation of infective persons and the observance of personal hygiene, but vaccinated groups may be expected to have a significantly lowered morbidity rate.

BCG vaccine is prepared by the Division of Laboratories and Research and is distributed for special use only. It is recommended particularly for persons who may be excessively exposed to tuberculous infection, such as nurses, medical students, physicians, and hospital personnel, inmates in mental hygiene hospitals, families of patients, and for selected population groups with high tuberculosis morbidity and mortality rates. Only persons who do not react to 0.01 mg. of Old Tuberculin or to 0.00002 mg. of PPD and who are not suspected of having tuberculosis should be vaccinated with BCG.

An exception may be made for newly-born infants of healthy parents.

Administration. The vaccine may be given by the multiple puncture methods, cutaneous scarification, or intracutaneous injection. The transcutaneous method is considered the method of choice; intracutaneous vaccination frequently produces a small abscess. Vaccine for the transcutaneous methods contains 20 mg. of microorganisms per ml.; that for the intracutaneous, 1.0 mg. per ml.

Do not combine BCG vaccination with vaccinations against other infectious diseases, such as smallpox, diphtheria, typhoid fever, or tetanus. If possible, vaccinated persons should be prevented from coming in contact with open or suspicious cases of tuberculosis for eight weeks after vaccination.

Multiple puncture methods. *Birkhaug's method.* Birkhaug's spring-actuated 40-needle instrument performs the multiple puncture vaccination with one downward action. A 4 x 4 cm. piece of thin paper or cellophane, sterilized for fifteen minutes at 100°C., is moistened on both sides with BCG vaccine in a sterile Petri dish and is placed on the ether-cleansed skin. The apparatus is cocked. With the skin held taut, the headplate is evenly pressed against the paper and the trigger is pushed. The needle plate descends and the needle-points become coated with the vaccine as they perforate the paper and enter the epidermis to a depth of 1 to 3 mm. The paper is removed after one or two minutes. No bandage is necessary over the vaccinated area. Pin-point bleeding may be seen in the punctures when the skin is stretched. Excessive spontaneous bleedings should be avoided; they can be controlled readily by adjusting the extension of the needles to 2 or 3 mm. beyond the headplate.

Rosenthal's method. With the arm or thigh in a horizontal position and the skin taut, 3 or 4 drops of vaccine are placed over the cleansed area and, with the side of the needle, the vaccine is spread evenly over an area of 3 x 4 cm. Twenty to forty pressure punctures are made in four or five rows, about ½ cm. apart. Sufficient pressure should be exerted for the needle-point to pierce the epidermis, but not to cause more than pin-point bleeding. With the skin still taut, the vaccine is rubbed gently over the puncture area with the side of the needle and allowed to dry for one to two minutes. No dressing is required.

Scarification method (Nègre and Bretey). Instead of puncturing the ether-cleansed area of the skin, three to four linear scarifications, 1 cm. long, are made across the drops of the 20-mg.-per-ml.

vaccine in infants and six to eight scarifications 2 cm. long in adults. The scarifications should be about ½ cm. apart. With the skin still taut, the vaccine is rubbed gently over the area with the side of the needle and allowed to dry for one to two minutes. No dressing is required.

The more numerous the punctures or scarifications, the more rapidly the tuberculin reaction becomes positive.

Intracutaneous method. The outer surface of the left upper arm or thigh is cleansed with ether or alcohol. With a sterile 1.0-ml. tuberculin syringe and a fine needle, preferably 26 gauge, 0.1 ml. of the vaccine is injected intracutaneously. Subcutaneous injection should be avoided. The beveled side of the needle should be upward.

Tuberculin reaction following vaccination. Successful BCG vaccination renders a person positive to tuberculin. It is important, therefore, to determine that the tuberculin reaction has become positive following vaccination. All vaccinated persons should be tuberculin tested intradermally two months after vaccination, using the same dose and substance that was utilized in determining original reaction to tuberculin. Tuberculin testing should be repeated every year thereafter for several years. The sensitiveness to tuberculin produced by BCG vaccination is such that it usually requires the use of a second dose of PPD or Old Tuberculin. The tuberculin test is considered positive if an edema or induration measuring 10 mm. in diameter is present 48 hours after the test has been performed. When the tuberculin reaction becomes frankly negative with 0.005 mg. of PPD or 0.1 mg. of Old Tuberculin, revaccination should be performed.

BCG vaccine should be used as promptly as possible after it is received, never more than ten days after its preparation. It should be kept in a refrigerator and not be used after the return date given on the label.

As soon as the actual number of persons to be vaccinated is ascertained, the required doses of BCG (a 5-ml. vial is sufficient for 15 to 20 vaccinations) should be ordered from the Division of Laboratories and Research, New Scotland Avenue, Albany 1.

Tularemia

Bacterium tularense, the incitant of tularemia, is acquired by man from an animal source. Rodents, especially rabbits, appear to be particularly susceptible, although many other species of animals, including birds, have been found to have the infection. *Bact. tularense* is transmitted from animal to animal and from animals to man by blood-sucking insects, as well as by direct contact in handling and dissection of animals. Transmission from man to man by con-

tact or by the bite of insects that have previously bitten a patient has not been reported.

Tularemia is relatively rare in New York State. Rabbits and other game animals native of the State have seldom been implicated, although muskrats in the northern and central sections have been indicated to be a source of infection.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, the following material should be submitted for examination to a laboratory approved for the purpose: (1) 10 ml. of blood (typhoid tube outfit); (2) if ulcerating lesions are present, films of discharge on glass slides (slide outfit), and a specimen of discharge on a sterile swab (tube outfit with swab). Dead animals or birds may also be submitted, in which case none of the organs should be removed.

Typhoid Fever and Other Salmonella Infections (including Paratyphoid Fever)

The present sanitary environment of urban districts in New York State is such that the incidence of typhoid fever is very low. The source of the incitant in most cases can be traced to carriers of typhoid bacilli (*Salmonella typhosa,* previously designated as *Bacterium typhosum*). Carriers who are food handlers represent a particular menace.

The Sanitary Code requirement (Chap. II, Reg. 15) which necessitates the submission of specimens from convalescents who have had typhoid fever and other *Salmonella* infections before release from observation, should result in the detection of most of the individuals who develop the carrier condition. Nearly all of these carriers have a focus of infection in the gall bladder. Gall stones or other evidence of cholecystitis are usually found when the gall bladder removed from a chronic typhoid carrier is examined.

In hospitals and other institutions, the ease with which incitants of enteric disease can be transmitted with the rectum as the portal of entry must be kept in mind. Improperly sterilized rectal catheters may be the means of transmission. Merely washing enema tubes or soaking them in an antiseptic is not an adequate protection, since the inside of the tubing may remain contaminated.

Typhoid fever. The results of serologic tests for evidence of typhoid fever are seldom of diagnostic value during the first week of symptoms. When the clots of blood are cultured, however, the incitant is usually isolated. After the patient has been ill for from ten days to two weeks, the blood usually agglutinates typhoid bacilli in high titer. Total and differential leucocyte counts are useful, since a leucopenia and the presence of a relatively high percentage of lymphocytes are characteristic findings in typhoid fever.

Specimens of feces collected a day or two after onset of symptoms may not be found to contain typhoid bacilli, but the microorganisms can usually be isolated from those collected later during the febrile phase of the illness and they may be present for a considerable time during convalescence. When these bacteria are found in specimens from a person who has not suffered from typhoid fever within one year, he is considered a chronic typhoid carrier (Sanitary Code, Chap. II, Reg. 31), and can be released only after compliance with the provisions of the Sanitary Code (Chap. II, Reg. 34).

Typhoid bacilli are present in the urine of a fairly high percentage of patients with typhoid fever. They are found also in discharges from focal infections and occasionally in cerebrospinal fluid and sputum.

If a cholecystectomy is considered desirable in order to free a typhoid carrier who has a focus of infection, duodenal contents should first be examined to prove that the bile contains these bacteria. Such specimens should also be studied after the gall bladder has been removed. Typhoid bacilli may be present in specimens of duodenal contents from three months to a year after removal of the gall bladder and yet the focus may later become inactive.

Since typhoid fever occurs so rarely in New York State, determination of the source of all infections is of special importance. An agglutination test with a so-called Vi strain of typhoid bacilli may be helpful in detecting typhoid carriers. In such a strain the special antigenic component designated as "Vi" predominates and those giving rise to granular (O) and floccular (H) agglutination are not demonstrable. This test can be applied to blood specimens from persons whose previous history, occupation or relationship to environmental factors suggests that they may have been the source of the incitant. While Vi agglutination may be obtained with blood from a small percentage of persons who are not typhoid carriers, the sera of a high percentage of typhoid carriers react. Hence, when "Vi" agglutination is obtained, fecal specimens should be examined

for typhoid bacilli. If *Salmonella typhosa* is isolated, bacteriophage typing of the culture provides a means of determining whether the strain found in the feces of the patient belongs to the same type as that isolated from specimens from the person believed to be the source of the incitant. If the strains are of different types, another source must be sought.

Paratyphoid fever and other Salmonella infections present problems similar to those encountered in typhoid fever. Certain diseases of rodents and domestic animals are incited by strains of *Salmonella* other than *S. paratyphi*-A and -B. Man is susceptible to infection with many of these microorganisms.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, the following material should be submitted for examination to a laboratory approved for the purpose: (1) 10 ml. of blood (typhoid tube outfit); (2) a specimen of fluid feces (typhoid jar outfit containing 30-per-cent buffered glycerol) and, if there is evidence of localization in the genitourinary tract, a specimen of urine (typhoid jar outfit containing 30-per-cent buffered glycerol). The 30-per-cent buffered glycerol inhibits fermentation and has thus proved efficient in preserving specimens of the type mentioned, when transmitted through the mail.

When the gall bladder of a carrier is to be removed, the following specimens should be examined:

Before operation. Three specimens of duodenal contents taken on three different days.

At the time of operation. (1) the gall bladder and its contents; (2) the appendix if it is removed; (3) about 10 ml. of blood for agglutination tests.

After operation. (1) Three specimens of the duodenal contents taken in a hospital at intervals of not less than twenty-four hours; (2) at least eight successive specimens of liquid feces and eight successive specimens of urine, taken on separate days, in a hospital or under other circumstances that do not permit of substitution. The patient cannot be released from the restrictions imposed on typhoid carriers until the series of specimens collected postoperatively has been examined in the laboratory of the State Department of Health and found to contain no typhoid bacilli. (Sanitary Code, Chap. II, Reg. 34.) It is provided, however, that if no "Vi"

agglutinative properties are found in the blood of the individual, he may be released from restrictions without removal of the gall bladder when the other requirements have been met.

The inconvenience to the patient occasioned by passing the drainage tube warrants the collection of at least three specimens of duodenal contents at 15- or 20-minute intervals so that at least one of them will be satisfactory. Only fluid that is neutral to litmus or very slightly acid or alkaline is usually satisfactory for cultural tests. When acid, the typhoid bacilli, if present, may not remain viable. The introduction of sterile sodium bicarbonate may be desirable to neutralize the acid. After the tip of the tube has reached the duodenum, administration of magnesium sulfate will promote the flow of bile and thus improve the opportunity for the detection of *Salmonella typhosa*.

Products Supplied by the Laboratory

Typhoid vaccine. The administration of typhoid vaccine has proved to be an effective preventive against typhoid fever. The duration of the protection afforded may be two years or possibly a considerably longer period. It should be borne in mind, however, that the immunity induced by this vaccine is only relative; it may not be sufficient to protect against frequent and massive doses of the infective agent. The vaccine is not recommended as a therapeutic agent to be used after the disease has developed.

Typhoid vaccine is prepared by the Division of Laboratories and Research and distributed through district laboratory supply stations in bottles containing three doses for the immunization of one person, and in larger bottles containing 10 ml. for use when a number of persons are to be immunized at the same time. Each milliliter of the vaccine contains 1,000 million killed bacilli.

Administration. The vaccine should be injected subcutaneously, usually over the insertion of the deltoid. Three doses are usually administered at intervals of from seven to ten days, the first dose being 0.5 ml., the second and third doses 1 ml. each. Although the dosage should not exceed the standard amounts recommended, slightly smaller doses may be given in order to reduce the severity of the reactions occasionally induced by the injections. The dosage for children should be reduced in proportion to the body weight as compared with that of an adult. For reimmunization, indicated under special circumstances, annual injections of 0.1 ml. intracutaneously or 0.5 ml. subcutaneously may be given.

The reaction induced by the vaccine varies; it usually consists of localized congestion with redness, swelling, and tenderness. These local reactions may be accompanied by varying degrees of systemic disturbance, general malaise, and fever. Pronounced systemic reactions are, however, relatively rare and are transitory in character. It is advisable to give the injections late in the afternoon so that if a reaction occurs it will be at night. The injections should be postponed in case of illness or after the fifth month of pregnancy. Great care should be exercised in cases of cardiac disease and nephritis—conditions which should be considered contraindications. The same is true of tuberculosis in the active stages, although typhoid vaccination in the latent or inactive stages is considered a safe procedure.

Full directions for dosage and administration are given in the circular that accompanies each bottle of vaccine.

Typhoid-paratyphoid vaccine. Because of the apparently low incidence of *Salmonella paratyphi* A and B infections in New York State, the general distribution of the combined typhoid-paratyphoid vaccine was discontinued in 1937. A small supply of typhoid-paratyphoid vaccine is, however, maintained for the immunization of special groups.

Undulant Fever

Until about twenty-five years ago, undulant fever was thought to be incited only by *Brucella melitensis* and to be restricted to districts where the milk of goats was used as a beverage. Man was believed to be immune to infection with *Brucella abortus,* the incitant of abortion disease in cattle, to which hogs are also susceptible. Investigation has proved, however, the close serologic relationship of the species of bacteria belonging to the Brucella group; all are pathogenic for man. In New York State the number of hogs and goats raised is not large and these animals are of negligible significance as sources of the incitant of undulant fever. In nearly all instances, patients with this disease have been found to have used raw milk from cows with infectious-abortion disease or to have handled such animals. The very small number of cases of undulant fever reported in the city of New York where all but a very small percentage of the milk is pasteurized and few of the residents handle cattle, indicates that butter, cheese, and uncooked meat that may be handled by the housewife do not represent a significant source of the incitant of undulant fever. Men in slaughter houses, however, who come in con-

tact with the tissues of diseased animals frequently acquire Brucellosis infection. A considerable percentage of patients with undulant fever develop foci of chronic infection; for example, cholecystitis or spondylitis may be incited by *Br. abortus,* or these bacteria may remain viable in an ovarian cyst.

The clinical manifestations of infections with members of the abortus-melitensis group of bacteria vary widely; in fact, cases may first be diagnosed as typhoid fever, malaria, influenza, tuberculosis, melancholia, or neurasthenia. Thus, the results of laboratory tests are of particular value. While the serum from most patients with undulant fever agglutinates *Br. abortus* in a 1:80 or higher dilution, no reaction or one of low titer only may be obtained early in the disease. When chronic foci develop, a marked reaction sometimes occurs, but in such cases low titers are not unusual.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, 10 ml. of blood (typhoid tube outfit) should be submitted for examination to a laboratory approved for the purpose. Blood may also be collected in citrate solution for cultural examination.

Note. A limited supply of *Br. abortus* vaccine is prepared and maintained by the Central Laboratory in Albany. While published reports and those received by this Division indicate wide variation in the results of vaccine therapy, the data accumulated appear sufficiently encouraging to warrant its use in suitable cases. Requests for the vaccine, made directly to the Central Laboratory, should give the significant facts concerning the case. Recommendations relating to dosage and administration are mailed to the physician.

Vincent's Angina

A diagnosis of Vincent's angina must be based largely on the clinical manifestations. The spirochetes and fusiform bacilli found in lesions of Vincent's angina are often present in other types of lesion in the mouth or throat. They are usually present in the necrotic material around the teeth of patients with pyorrhea. Films from the surface of the membrane in diphtheria may be found to contain large numbers of fusiform bacilli and spirochetes. Hence, the presence of these microorganisms requires careful evaluation.

Specimens for Laboratory Examination

In accordance with the requirements of Chap. II, Reg. 9, of the Sanitary Code, the following material should be submitted

for examination to a laboratory approved for the purpose: (1) films of the exudate on glass slides (slide outfit); (2) a culture from the exudate on Loeffler's blood-serum medium, to be examined for diphtheria bacilli and hemolytic streptococci (diphtheria culture outfit).

Whooping Cough

Laboratory aids in the diagnosis of whooping cough are unnecessary when the patient has characteristic symptoms. In the case of children who have not developed the "whoop," or adults in whom the manifestations may not be typical, bacteriologic findings may be very helpful. *Hemophilis pertussis* can be isolated in the great majority of cases during the first two or three weeks of the disease by the cough-plate or nasopharyngeal-swab method.

A special medium is necessary for the isolation of *H. pertussis.* The work can best be done in a nearby laboratory. When an examination for the presence of *H. pertussis* is desirable, the patient is induced to cough on suitable medium in a Petri plate. In small children, examination of cultures from the nasopharynx will often reveal the microorganism.

Products Supplied by the Laboratory

Pertussis vaccine. Pertussis vaccine is used as a prophylactic agent. Experience indicates that phase-I vaccine has definite preventive value against whooping cough if administered before exposure to the disease and that, when cases occur in vaccinated individuals, the course of illness is frequently modified. Local reactions to the vaccine may be expected but disturbing systemic reactions are rare.

The vaccine prepared by the Central Laboratory is distributed through district supply stations in bottles containing 5 and 20 ml. The vaccine contains 20,000 million microorganisms per milliliter. It should be kept at a low, even temperature.

Administration. The vaccine is injected subcutaneously. Just before the vaccine is withdrawn, the bottle should be vigorously shaken to make sure that the bacilli are suspended evenly in each dose. Three injections are usually given seven to fourteen days apart. Full directions for dosage and administration are given in the circular contained in each package.

Antipertussis serum. Favorable results have been obtained in the serum therapy of whooping cough, especially in the early stages

of the disease. There is also evidence that the administration of the serum to infants exposed to whooping cough may prevent an attack or modify the course of illness.

Antipertussis serum (rabbit), concentrated and purified, is prepared by the Division of Laboratories and Research. It is supplied through certain of the district laboratory supply stations in vials containing 10 ml. for the prophylaxis and treatment of whooping cough in children under two years and for the treatment of children over two years who are critically ill.

Administration. The serum should be administered intramuscularly. For prophylaxis, one dose of 5 or 10 ml. is generally sufficient. For the treatment of infants under three months, 5 or 10 ml. of serum may be given followed by a second dose in 24 or 48 hours. For children over three months, two 10-ml. doses should be administered. Additional treatment is rarely necessary but if, after several days, it seems desirable, due regard must be given to possible serum sensitivity. A circular of directions is contained in each package. For precautions against anaphylactic reactions, see page 19.

Miscellaneous Examinations

Microscopic, cultural, and chemical examinations of blood, urine, and other body fluids, secretions, excretions, exudates, and transudates, and histologic examination of tissues may furnish valuable aids in the diagnosis, prognosis, and subsequent treatment of many types of disease processes. The local laboratory is in the best position to make these examinations since, in most instances, proximity to the patient is an important factor.

Blood

Chemical examination. The chemical examination of blood is not undertaken at present as a routine procedure either in the Central Laboratory or in the Branch Laboratory, but is included in the service rendered by most of the approved laboratories. When planning to have such tests performed, the physician should first consult the director of the laboratory concerning methods for the collection and submission of specimens.

Cultural examination. While the typhoid bacillus can often be isolated from the blood clot after the specimen has been in transit for a day or more, cultural examination of the blood in most types of bacteriemia should be undertaken promptly after collection of the

specimen. Experience has demonstrated that in addition to the usual aerobic procedures, anaerobic methods and provision for from five to ten parts of carbon dioxide in the atmosphere in which the cultures are incubated, adds materially to the value of the work. Also, the opportunity to isolate the inciting microorganism is increased if at least 10 ml. of blood are cultured. The use of ten parts or more of culture medium to one part of blood is desirable. (See p. 16).

Human blood donors. Laboratory tests required as an aid in determining that blood donors are free from communicable disease, including malaria and syphilis, and tests of sterility required to determine that the blood, plasma, serum, or any derivatives of them is suitable for therapeutic or prophylactic purposes shall be made in a laboratory approved for such examinations by the state commissioner of health, in a laboratory licensed by the United States Public Health Service for the preparation of human blood, plasma, serum, or other human blood derivatives, or in a laboratory maintained by the United States Army, Navy, Veterans Bureau, or Public Health Service.

In every case, a specimen of blood shall be collected from the donor at the time of transfusion and sent to a laboratory approved for serologic tests for evidence of syphilis. If, due to an emergency, a specimen of the donor's blood cannot be sent to an approved laboratory prior to the transfusion of blood, a preliminary test for evidence of syphilis shall be made. (Sanitary Code, Chap. IV-A.)

Microscopic examination and hemoglobin determination. With the exception of the examination of dried films, none of the laboratory aids in diagnosis that can be furnished by studies of the cellular elements and platelets in the blood is successful if the material is submitted by mail. Recent investigations in this field emphasize the importance in diagnosis and prognosis of the number and condition of the various types of cells found in the blood and their relationship to the hemoglobin content. Here again, the director of the local laboratory is in an advantageous position to assist the clinician and the surgeon in his district.

Urine

Chemical and microscopic examinations. Since no preservative has been found entirely satisfactory in preventing decomposition in urine, chemical and microscopic examinations are essentially procedures that should be done in a local laboratory. The urine should be collected in a clean receptacle, free from acid or alkalis,

and sent to the laboratory promptly. For quantitative sugar determination, a portion of a 24-hour specimen should be submitted.

Cultural examination. In case of infections of the urinary tract, a specimen collected aseptically, preferably by catheterization, should be examined promptly after it has been obtained.

Antibiotics and Sulfonamides

The therapeutic value of penicillin, streptomycin, certain other antibiotics and the sulfonamides has been proved, and for many species general agreement has been reached as to those that are susceptible or resistant to their action. It has been found, however, that some strains of species usually susceptible to antibiotics and sulfonamides may become resistant. Hence examinations to determine the susceptibility of a strain may be important. Work of this type can be undertaken most advantageously in a local laboratory.

Autogenous Vaccines

Isolation of cultures. Since the first requisite for an autogenous vaccine is a pure culture of the incitant of the lesion, the cultural examination should be undertaken promptly after collection of the specimen to obviate the possibility of overgrowth by contaminating microorganisms and of destruction of the pathogenic species.

Autogenous vaccines are not prepared as a routine procedure by the Division of Laboratories and Research. Since the etiologic relationship of a particular microorganism to a subacute or chronic infection is often difficult to establish from bacteriologic study alone, work of this type, when required, should be undertaken in a local approved laboratory.

PART III

EXAMINATION OF WATER, SEWAGE, AND SEWAGE SLUDGE

Water

Samples of water are examined at the request of directors of the divisions of the State Department of Health, district state health officers, district sanitary engineers, county health commissioners, and local health officers. Individuals are referred to the local health officer; if in his judgment examinations to determine sanitary quality are desirable, they are made, but only if he collects the samples in containers supplied by the laboratory, and furnishes a record of the sanitary conditions at the source of the supply. Samples for laboratory examination should not be collected from sources where the surroundings are obviously insanitary until the condition has been corrected. Samples from private sources are examined only for the sanitary quality of the water; for mineral analyses the owner is referred to a private laboratory for a special study of the particular problem.

The health officer should state his reason for requesting a laboratory examination in his investigation of a water supply, and also the number of samples it is proposed to collect for chemical and bacteriologic examination. Since a single bacteriologic examination may not reveal intermittent pollution, a sample for chemical analysis should be collected from all privately owned sources where previous examinations have not been made. When the health officer is concerned with problems in the operation and supervision of a public water supply or in the investigation of the source of water serving schools, institutions, or summer resorts, he should consult the district sanitary engineer or, in a county health unit, the county sanitary engineer, who will advise him and arrange for the collection of the necessary samples.

When the health officer collects samples for examination in the containers from this Division, it is essential that he answer all questions relating to the conditions found in his inspection on the forms provided.

The laboratory examination determines the presence or absence of pollution at the time of sampling, but the field inspection determines the sources and nature of the pollution and thus the significance of its presence or absence. Reports are not made unless the sources of supply have been adequately inspected. If the descriptive forms are incomplete, they will be returned and the report of the examination held until the necessary data are furnished.

Ground waters receive pollution from surface washings, from subsurface drainage through the soil, or through fissures and channels in rock strata. It is, therefore, necessary to inspect the well, spring, or infiltration gallery, and to record data to show: (1) whether the well is protected structurally from pollution; (2) the nature and location of, and drainage from nearby sources of pollution; and (3) the character of the soil penetrated. The location of all potential sources of pollution should be noted. If sewage disposal is by means of septic tank, the location of the drainage area or tile field in relation to the well should be indicated on the descriptive card. Although information concerning the geologic formations penetrated by the well may not be available, frequently an examination of the surface conditions will indicate the probable presence of rock strata, or in the case of any but deep driven wells, that the soil penetrated is either silt, gravel, clay, or loam.

Surface waters receive pollution at different points from various sources and through tributaries. This pollution is altered by storage and by sedimentation, dilution, and many other natural agencies.

Treated waters. Various methods of purification and different combinations of these methods are used in the treatment of water. Since the efficiency of any method, or combination of methods, is dependent upon the precision with which each step of the process is carried out, it is necessary to record the operative details, all of which can be obtained from the person in charge of the treatment plant.

The mailing outfit for the small sample for bacteriologic examination is sent to the health officer by parcel post; the one-gallon bottle for the sample for chemical analysis is shipped by express collect. The two samples should be collected at the same time and returned to the laboratory on the same day, charges prepaid. Samples, bacteriologic and chemical, from different points in a source of supply, should be identified by the letters A, B, C, D, etc., the respective points at which these samples were taken being noted on the des-

criptive cards. The sampling schedule should be timed, and direct shipping routes selected to ensure delivery within twenty-four hours, if possible, but never more than forty-eight hours after collection. Samples should be taken preferably early in the week and not later than Thursday.

Swimming Pools and Bathing Areas

Samples of water from swimming pools and bathing areas are ordinarily examined only when submitted by a member of the Department staff in connection with a special study. If a health officer has any problem concerning supervision and operation of swimming pools or bathing areas, he should consult the district or county sanitary engineer for advice and assistance in the investigation. A sterile bottle containing a dechlorinating agent is supplied for the collection of samples from swimming pools. Any excess chlorine in the sample is thus neutralized and the examination will indicate the bacterial content of the water more nearly than if the residual chlorine were allowed to remain in the sample during transit. The bottles are distinguished by the fact that they have a protective cover of paper instead of metal foil and cloth.

Public Water Supplies

As an aid in supervision and to provide data on which the Division of Sanitation may base recommendations for improvement, samples from all public water supplies are examined at scheduled intervals. Chemical examinations are made at least annually; bacteriologic examinations four, six, and twelve or more times a year. In general, supplies from surface sources, or those in which treatment is essential for maintenance of sanitary quality, are sampled at monthly or even shorter intervals. The frequency of sampling depends upon the results of previous examinations and upon known sanitary and operating conditions. When local approved county or city laboratory service is available and reports of monthly or more frequent bacteriologic examinations at these laboratories are submitted to the Department, the supplies are listed for minimum sampling. Occasionally, the frequency of sampling is increased temporarily. Included in this routine sampling schedule are the water supplies of state institutions and state parks, over which close supervision is necessary in the interests of the public health. Large central schools served by individual water supplies and other schools in which the supply is a potential public

health hazard have been selected for sampling at two-month intervals during the school year.

Samples from public supplies examined under this schedule are shipped without refrigeration by parcel post, special delivery, in an outfit of special design; in this way delivery within twenty-four hours is assured. Experience has indicated that in water not seriously polluted bacterial changes during unrefrigerated storage for twenty-four hours are of little significance. Samples without special delivery postage are not examined and another specimen is requested from the same source.

The Central Laboratory ships the sampling outfit to the health officer or water department official according to the established schedule, advising him at the same time by postal card when the sample should be submitted. The sample should be collected as nearly as possible on the designated sampling date and should be submitted in the container furnished for that specific purpose. If the sample is not received within a week, a postal card is sent to the district engineer requesting that he investigate the cause of delay.

The outfit includes a card on which all identifying and descriptive data regarding the sample and the supply should be entered. In order to avoid confusion, the name of the supply sampled should be written on the descriptive card as it appears on the postal card notice. If the water is chlorinated, the residual chlorine value on the day of sampling should be recorded, since it is essential to an interpretation of the laboratory findings. This information can be secured readily from the operator in charge of the treatment. Incomplete cards are returned for detailed information before the results are reported.

The laboratory findings are sent to the Division of Sanitation for interpretation and submission to the local officials.

The information furnished by this sampling procedure has been of great value in the control of water quality and in the establishment of more satisfactory public supplies throughout the State. When the laboratory results indicate that the sanitary quality of a supply is questionable, an inspection is made promptly by a member of the staff of the Division of Sanitation.

Samples for bacteriologic examination. Samples should represent water in the distribution system and not the source of the supply. Taps on a main that is in constant use should be selected as sampling points; never those on a dead end. Leaky taps and hydrants are not suitable as sampling points. The water should be allowed to run

for ten minutes before samples are taken. The bottles for bacterial samples are sterile and should be handled with care to avoid contamination. If by accident a bottle should become contaminated, or there is any question of its sterility, it should be so marked and returned to the laboratory and a fresh one taken or secured from the Central Laboratory if not available locally.

Method of collection: Wash and dry the hands carefully. With one hand hold the bottle at or near the bottom; with the other, loosen the string around the cap and remove the stopper with the protective paper cap in place. A short piece of string is inserted between the glass stopper and the neck of the bottle to facilitate removal of the stopper. Do not replace string in the bottle when the sample has been collected.

The sterile bottles for the collection of samples from public water supplies contain a dechlorinating agent to neutralize any residual chlorine and thus to ensure bacteriologic findings that are truly indicative of the condition of the water at the sampling point. The dechlorinating agent may appear as a white powder or white specks on the inside of the bottle. These bottles are identified by having a paper cover cap over the stopper; they should be used only for the collection of samples from public water supplies. Samples from other sources should be collected in bottles which do not contain a dechlorinating agent and which are identified by a metal foil and muslin cap.

While collecting the sample, be sure that the exposed stopper does not touch anything, that the neck of the bottle is not contaminated by the hands, and that the water does not flow over the hands into the bottle. Fill the bottle to within half an inch of the stopper, leaving only sufficient air space for expansion. Replace the stopper and tie the hood down securely.

Label each sample with an identifying letter and the name of the city, town, or village, and enter the corresponding identification on the descriptive card of inspection. When from two to six samples from a single source are submitted for bacteriologic examination, it is more convenient to mail them in the special carton provided for the purpose.

Samples from newly constructed or recently cleaned wells should be identified as such; at least a week should elapse between the time water is first pumped and the collection of the samples. The well should be pumped frequently during the interim. From five to ten

pails of water should be pumped before a sample is collected. If there is any overflow or splashing back into the well during sampling, this fact should be recorded. Pails or buckets that are used for taking samples from wells or springs should be carefully cleaned and thoroughly rinsed with boiling water.

In ponds, reservoirs, or streams, samples should be taken in a sufficient depth of water to avoid disturbing the sediment or otherwise affecting the usual conditions. Grasping the bottle in the right hand near the bottom, plunge it mouth downward well under the surface, keeping the hand on the downstream side of the bottle; then carry the bottle upstream under the surface and out of the water, all in one continuous motion. Great care should be taken to avoid having the water flow over the hand into the bottle. The bottle should be plunged quickly below the surface and removed quickly to prevent entrainment of any surface scum. In rapidly flowing water the bottle may be held upstream and allowed to fill in this manner.

Samples for chemical analysis. The large bottle for the chemical sample is clean but not sterile. It is shipped by express in a wooden box with inner side spring to hold the bottle firmly in place and protect against breakage. Selection of sampling points should be made with the same care as in the collection of bacterial samples.

The bottle should first be rinsed with the water to be collected, then filled with the sample, and precautions taken against the entrance of foreign material. If possible the sample should be collected directly, without the use of a pail, dipper, or funnel. If such apparatus is necessary it should be clean and thoroughly rinsed in the water to be sampled. Unless otherwise directed, the bottle should be filled to within two inches of the stopper, leaving only sufficient air space for expansion. The stopper should be kept free from contamination as in taking the bacterial samples. If by accident the stopper of the bottle should become soiled, it may be washed thoroughly in the water being sampled and replaced in the bottle. The stopper and neck of the bottle should be recovered with the cloth and tied securely. The ends of the string may then be sealed but not the stopper. The bottle should be labeled with the name of the city, village, or town and the letters A, B, C, etc., to conform with the identification of the bacterial sample collected from the same source. Care should be taken to enter the proper descriptions of these samples on the cards, on which are recorded the results of the sanitary inspection.

Sewage

Samples of sewage, sewage effluents, and industrial wastes are examined only when submitted by members of the Department staff in investigations of the operation of sewage treatment plants or of stream pollution. When problems concerned with sewage treatment or disposal, stream pollution, or other nuisance conditions are brought to the attention of the local health officer, he should immediately consult the district sanitary engineer for advice and assistance in any investigation which may seem necessary. Samples of sewage must be accompanied by the necessary identifying and descriptive data regarding the source, type of treatment, and method of plant operation at the time of sampling, and the purpose of the investigation

Bacteriologic examination. "Catch" samples should be taken in the type of sterile bottle used for the collection of samples of water for bacteriologic examinations. Samples of chlorinated sewage effluents should be collected in sterile bottles containing sodium thiosulfate to neutralize the residual chlorine in the sample. All samples must be carefully refrigerated from the time of collection and delivered to the laboratory as soon as possible.

Chemical analysis. Sampling points should be so located as to permit the collection of representative samples. Precautions against contaminating the sample with floating scum or sludge should be observed. Samples should be composites of specimens taken at intervals of not more than two hours, preferably more frequently, over a period determined by the nature of the investigation; when possible they should be integrated according to the rate of flow of the sewage. They should be submitted in duplicate in the glass-stoppered bottles (1 gallon capacity) furnished by the laboratory; concentrated sulfuric acid, C.P., (specific gravity 1.84) in the proportion of 1 ml. to 1 liter should be added as a preservative to one sample, and 5 ml. of chloroform per liter, to the second. If the biochemical oxygen demand is to be determined, a third sample without preservative should be submitted. Samples must be refrigerated from the time of collection and delivered to the laboratory as soon as possible.

Sewage Sludge

Chemical analysis. Samples of sewage sludge are examined only when submitted by members of the Department staff in investiga-

tions of the operation of sewage treatment plants. They should be submitted in wide-mouthed, glass-stoppered bottles or in preserve jars, and must be accompanied by complete identifying and descriptive data regarding the source of both sewage and sludge and the type of treatment at time of sampling. Methods that ensure the collection of representative samples must be used. Samples must be refrigerated from the time of collection and delivered to the laboratory as soon as possible.

PART IV

EXAMINATION OF MILK, CREAM, OR MILK PRODUCTS

Samples of milk, cream, or milk products are examined when submitted by the district milk sanitarians of the Bureau of Milk Sanitation in connection with the supervision of pasteurizing plants and the control of milk sanitation. Similar assistance is given to county health units upon request. The local health officer requesting examinations to assist in carrying out the provisions of the Sanitary Code (Chap. III, Reg. 5) is referred to a local approved laboratory.

Sampling of milk and cream. Before sampling, milk or cream should be thoroughly stirred with a sterile rod or by inverting the container several times. Samples of at least 15 ml. should be collected through sterile glass or metal tubes of a length sufficient to reach the bottom of the original container, and transferred to sterile screw-capped vials or glass-stoppered bottles protected against subsequent contamination and leakage. Containers should be not more than two-thirds full in order to permit adequate agitation before portions of the sample are removed for examination. Bottled milk should be submitted in the original unopened container as distributed by the dealer.

All samples should be packed in sufficient cracked ice to ensure constant refrigeration during transportation to the laboratory. Special insulated shipping cartons providing for refrigeration by ice are furnished for the use of the district milk sanitarians. Shipment is made by parcel post, special delivery, to ensure delivery of the specimen at a temperature less than 50° F. Samples should be accompanied by a record of the identification marks on the original container, the source—name and location of the dairy, bottling plant, creamery, producer, or distributor—the grade, whether raw or pasteurized, the date and time of collection and of shipment, the examination requested, and any other pertinent information.

Bacteriologic Examination

The standard agar plate count provides information of value in the routine control of the sanitary quality of a milk supply and

is essential to determine compliance with the standards of grading given in the Sanitary Code.

Direct microscopic examination detects unclean milk and that from cows with diseased udders. Since it yields valuable information concerning the bacterial and cell content and may reveal the presence of flora introduced in processing, this method should be used in conjunction with the agar count on all samples, both raw and pasteurized. The direct microscopic count is not satisfactory for the accurate grading of Certified or other grades of raw milk with low counts; marked deviations from these grades can be determined, however, and it is, therefore, a desirable auxiliary examination.

Test for coliform bacteria. Under the usual conditions of production and handling, milk or cream may become contaminated with bacteria of the coliform group. Since these microorganisms multiply in such a favorable milieu, their determination in raw milk is of little value. They are destroyed at the temperature of pasteurization and their presence in pasteurized milk thus indicates subsequent contamination by faulty handling. If the density is greater than 10 per milliliter the contamination warrants immediate remedial measures. The sanitary quality of all pasteurized milk and cream should, therefore, be checked by this examination.

Test for Pasteurization

The addition of significant amounts of raw milk to a pasteurized product cannot always be detected by bacteriologic examination, nor can variations in pasteurizing treatment. A laboratory procedure to detect irregularities of this character is a valuable aid in the control of the processing of milk. The phosphatase test detects almost without failure a lowering of as little as 1° F. in the temperature of pasteurization, shortening of the holding time by five minutes, and the addition of more than 0.1 per cent raw milk. It is thus exceedingly valuable in the control of pasteurization. The laboratory routinely checks against the possibility of faulty pasteurization by making a phosphatase test on all samples of pasteurized milk submitted for bacteriologic examination.

In summary, the routine supervision of milk supplies should include the standard agar plate count and the direct microscopic count on all samples, both raw and pasteurized; and, in addition, the test for coliform bacteria, and the phosphatase test on all pasteurized products.

The results of the examination of a single sample of milk or cream often do not fairly represent the character of a supply. If a particular sample has a high standard plate count and a phosphatase value indicative of inadequate pasteurization, or shows the presence of significant numbers of bacteria of the coliform group, an investigation should be made to discover the cause. Additional samples should be examined to determine whether the results were indicative of a single faulty condition or whether the entire supply is intermittently or constantly below standard.

Determination of the butterfat content, solids, and preservatives in milk or cream is not made by the Division of Laboratories and Research, since the laws and regulations that relate to food value and adulteration are under the direction of the Department of Agriculture and Markets.

Bio-assay of Vitamin D Milk

Regulation 1, Chapter III of the Sanitary Code requires that Vitamin D milk shall be examined semiannually in a laboratory approved for the purpose. Dealers who supply Vitamin D milk for sale are required to submit specimens for bio-assay to one of a group of laboratories that specializes in this determination and has been approved by the State Commissioner of Health.

Examination of Baby Feeding Formulae

The preparation of baby feeding formulae as dispensed in hospital nurseries is subject to the requirements of Sanitary Code Chapter II, Regulation 35 (effective January 1, 1949). Although no bacterial standards are established by the regulation, methods for laboratory examination of sterilized formulae have been developed for use in laboratories approved for this examination.

Baby formula milk prepared, offered for sale, sold, or delivered is subject to the requirements of Chapter III-B, Regulations 1-14 of the Sanitary Code. Regulation 10 specifies that the product shall be sterile upon bacteriologic examination made in a laboratory approved for the purpose.

PART V

EXAMINATIONS CONCERNED WITH EATING, DRINKING, AND COOKING UTENSILS

A standard of 100 microorganisms per utensil surface area is established in the Sanitary Code (Chap. XIV, Reg. 3) as a criterion of the satisfactory cleansing of eating, drinking, and cooking utensils. Investigation has shown that adequate washing in water at 120° - 140° F. in which there is a suitable detergent, followed by adequate rinsing in water at 170° F. or greater will satisfy this standard; and, further, that if such utensils are protected subsequently during storage against contamination by handling, they will be free from bacteria of the coliform group.

Laboratory examination of these utensils, particularly of glassware, is usually not necessary, since the methods of washing and rinsing and the general cleanliness of an establishment ordinarily indicate whether the regulations of the Code are met. Occasionally, it is necessary to demonstrate that specific methods of washing and handling produce or fail to produce results that meet the standard, or to provide evidence regarding compliance with the regulations. When such examinations are required, the general sanitation of the establishment and information regarding the specific methods of washing and rinsing are necessary to an interpretation of the results, and should be furnished to the laboratory. The Code specifies that such examinations must be made in a laboratory approved for the purpose.

PART VI

RECORDING AND REPORTING RESULTS OF LABORATORY EXAMINATIONS

The maintenance of accurate records of specimens received and the prompt and correct reporting of results of examinations are procedures of the utmost importance.

To facilitate handling and to eliminate any possibility of interchange in the laboratory, only one specimen is opened at a time; it is given a serial number before another container is opened. As a safeguard against loss or misplacement of specimens, accession books are kept for recording their receipt and pertinent data concerning them. These books are also the source of statistics used in the compilation of monthly and annual reports.

Diagnostic Laboratories. Typed reports of all examinations are sent to the physician by whom the specimen is submitted and, if the examination discloses the existence of a communicable or malignant disease, to the local or state health official to whom the physician is required to report the case (Public Health Law, Art. III, Sec. 25 and 25-b). A similar procedure is required (Public Health Law, Art. III, Sec. 25) when results of examinations are needed for purposes of release from quarantine or observation.

Copies of reports are sent to hospitals for purposes of record, if the physician makes such a request on the history form.

While the interests of the patient are considered in all cases where there is occasion to divulge information in regard to laboratory examinations, the Public Health Law (Art. III, Sec. 25) and the Sanitary Code (Chap. II, Reg. 26) require all records relating to suspected cases of syphilis, gonorrhea, or chancroid to be treated as strictly confidential. Copies of reports are therefore given only to health officials and to the physician by whom the specimen is submitted, unless the patient furnishes a waiver stating to whom he wishes the information furnished.

The Public Health Law (Art. III, Sec. 25, and Art. XVI, Sec. 322) and the Sanitary Code (Chap. II, Reg. 28-29) require records relating to cases of tuberculosis to be considered confidential also.

Laboratories for Sanitary and Analytical Chemistry. Reports on the examination of water from public and institutional supplies are submitted to the Division of Sanitation for transmission to the local officials concerned. Reports on the examination of samples of water, milk, restaurant utensils, and sewage, submitted by the Division of Sanitation, are forwarded to that Division. Samples submitted from private supplies are reported to the health officer who collected them and to the district state health officer.

PART VII

DISTRIBUTION OF LABORATORY SUPPLIES

Article II, Section 5, of the Public Health Law empowers the State Commissioner of Health or his authorized representative to establish district laboratory supply stations, to prescribe the district to be served by each, and to appoint a custodian to have charge of each station. A health officer or person in charge of a public health laboratory or, when necessary, some other competent person may be appointed. The law also authorizes the establishment of substations by the custodians upon approval of the State Department of Health. Substations should be established only in large cities and in counties where there is a county laboratory or a county health unit. All other stations should be main stations. If substations are essential for the satisfactory distribution of supplies to physicians in such cities and counties, they should be established only with the approval of the Central Laboratory. The substations should be located where there are facilities for keeping supplies under proper conditions and should be placed in charge of qualified persons. The fee received for each substation is payable to the custodian of the substation.

The law provides that each custodian of a main station shall be entitled to receive annually certain fees and the actual and necessary expenses for maintaining and operating the district station and its substations, upon certification of the State Department of Health that such stations have been maintained and operated in accordance with its rules and regulations.

District supply stations have been established throughout the State, and laboratory supplies, including outfits for diagnostic specimens and prophylactic and therapeutic preparations, with a few exceptions, are distributed through these district stations and their substations.

Health officers and physicians should secure supplies required for immediate use from the station that serves the municipality in which they are located. In an emergency they should be procured from the nearest station. Laboratory supplies, especially the perishable products, should not be kept in quantity except in regularly established stations. If difficulties are experienced or delays occur, the matter should be taken up with the district custodian or, if necessary, with the Division of Laboratories and Research.

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